Scientific Program
BiodosEPR-2006

The 2nd International Conference on Biodosimetry and
7th International Symposium on EPR Dosimetry and Applications

Uniformed Services University of the Health Sciences (USUHS)
Bethesda, Maryland, USA
10-13 July 2006

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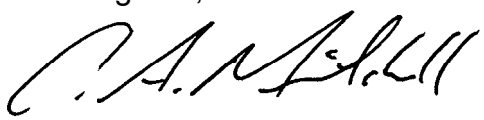
Dear Colleagues,

It is a great honor to welcome you to BiodosEPR-2006!

Due to recent world events, interest in retrospective dosimetry is surging. As such, it is appropriate that the format of the meeting include other forms of biodosimetry and emerging technologies as had been done in the past. Further, every effort has been taken to include national and international representatives in order to promote ongoing dialog between scientists and policymakers.

I would like to thank everyone who helped make this conference a reality. Their contributions are listed on the following pages. We certainly hope that the Uniformed Services University of the Health Sciences (USUHS) provides an atmosphere that is conducive to the exchange of ideas.

Best Regards,



Lt. Cmdr Chad A. Mitchell, Ph.D., DABR
Medical Service Corps, United States Navy
Chair, Organizing Committee BiodosEPR-2006

Conference History

The table below shows the history of the International Symposium on Electron Spin Resonance (ESR) Dosimetry. During this time, worldwide use of Electron Paramagnetic Resonance (EPR) dosimetry (ESR and EPR are often used interchangeably) has increased in disaster planning, and studies sponsored by IAEA and ICRU have demonstrated its' usefulness in evaluating both long-term and short-term exposures. It is important to bring this progress together with other forms of retrospective biodosimetry the joint format proposed for BiodosEPR-2006.

Conference Name	Dates	Location	Proceedings Published
1st International Symposium on ESR Dating and Dosimetry	1985	Ube, Japan	*ESR Dating and Dosimetry,* (*Proceedings of the First International Symposium on ESR Dating*), Ube, Japan (Ionics, 1985).
2nd International Symposium on ESR Dosimetry and Applications	10-13 Oct 1988	Munich, Germany	International Journal of Radiation Applications and Instrumentation A
3rd International Symposium on ESR Dosimetry and Applications	14-18 Oct 1991	Gaithersburg, Maryland, USA	Applied Radiation and Isotopes
4th International Symposium on ESR Dosimetry and Applications	15-19 May 1995	Munich, Germany	Applied Radiation and Isotopes
1st Joint International Conference on Biodosimetry' and 5th International Symposium on ESR Dosimetry and Applications	22-26 June 1998	Obninsk, Russia	Applied Radiation and Isotopes
6th International Symposium on ESR Dosimetry and Applications.	12-16 Oct 2003	Campos do Jordao, Brazil	Applied Radiation and Isotopes

The specific aims of this conference are to

- Provide a forum for showcasing the relative merits and synergistic aspects of the wide variety of techniques from physical techniques (EPR, optically stimulated luminescence) to biological techniques (cytogenetic aberrations, protein expression, etc)
- Disseminate recent advances in existing techniques and new applications
- Share results of laboratory intercomparisons and standardizations
- Spread 'lessons learned' during disaster response planning from policymakers and government scientists from around the world.

- Generate two consensus documents and vet them before the conference in order to provide policymakers and researchers with state of the art information on the use and utility of dosimetric techniques with respect to
    - Dosimetry for long-term, epidemiological studies
    - Acute dosimetry for radiological/nuclear accidents or terrorist attacks

Committees

Organizing Committee:

Chad A. Mitchell, Ph.D., DABR (Chair)	Uniformed Services University of the Health Sciences (USUHS)
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Alexander Romanyukha, Ph.D.	USUHS
David A. Schauer, Sc.D., CHP	National Council on Radiation Protection and Measurements (NCRP)
Anne Skinner, Ph.D.	Williams College

Program Committee:

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William F. Blakely, Ph.D.	AFRRI / USUHS

Local Events/Administrative Support:

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Shin Toyoda, Ph.D.	Okayama University of Science, Okayama, Japan
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Diane Wilkinson, MD	Defence R&D Canada, Ottowa, Canada

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Ruth Kleinerman, Ph.D.	National Cancer Institute, Bethesda, MD
Albrecht Wieser, Ph.D.	National Research Center for Environment and Health, Munich, Germany
Paola Fattibene, Ph.D.	Istituto Nazionale di Fisica Nucleare, Rome, Italy
James Tucker, Ph.D.	Wayne State University, Detroit, MI
Alexander Sevan'kaev, Ph.D.	Medical Radiological Research Center, Obninsk, Russia
Steve McKeever, Ph.D.	Oklahoma University, Stillwater, OK
David Lloyd, Ph.D.	Health Protection Agency, London, UK

Consensus Committee 2: Dosimetry Applications in Events Involving Terrorist Uses of Radioactive Materials and Radiation Accidents

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Consensus Committee 2 (continued)

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Government Agency Support

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Department of Health and Human Services
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Armed Forces Radiobiology Research Institute / USUHS
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Corporate Support

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Scientific Program

Monday, 10 July 2006

	Session	Speaker	Title	Page
8:00	**Registration**			
8:30	**Welcome**	Dr. Anne Skinner		
8:40	**USUHS Welcome**	Dean L. W. Laughlin, MD, Ph.D.		
8:45	**Announcements**	LCDR Chad Mitchell		
9:00	**Consensus Sessions Introduction**	Tenforde	Consensus Sessions: Structure & Purpose	
9:15	**Keynote Talks Chair: Dr. Tenforde**	Ramakrishnan	NIAID - CMCR Inititative	S-1
9:30				
9:45		Regulla	ESR – Global Player in Dosimetry	S-2
10:00				
10:15	**Morning Break**			
10:30				
10:45	**Session A: Fundamentals of EPR Dosimetry**	G. Eaton (Plenary Speaker)	Electron Spin Relaxation Times of Radiation-Induced Defects in Teeth	A-1
11:00				
11:15	**Chair: Shin Toyoda**	Nagy	Accuracy Considerations in EPR Dosimetry, Part 2—Regression-Related Aspects	A-2
11:30		S. Eaton	Rapid-Scan EPR: A New Magnetic Resonance Method with Potential Application to Dosimetry	A-3
11:45		Hyrien	Profile likelihood for dose estimation in radiation dosimetry studies	A-4
12:00	**Lunch & Posters**	Poster Session 1	Dating: Dolo, Teng	P-1,2
12:15			Tooth Enamel: Borysheva, Egersdörfer, Güttler, Rudko, Sholom, Sullasi, Toyoda, Wieser	P-3..10
12:30			Alanine: Chen, DeAngelis, LeBlanc	P-11..13
12:45			New Materials: Borgonove, DaCosta Ludwig, Tatumi, Melo	P-14..18
13:00			New Technologies: Grinberg, Hirata, Kmiec, Pass, Punchard, Sucheta	P-19..24
13:15				
13:30	**Session B: Archaeological and Geological Dating**	Toyoda	Tribute to Motoji Ikeya	B-1
13:45	**Chair: Dieter Regulla**	Skinner	ESR Analyses for the Paleolithic Hominid Site at Obi-Rakhmat, Uzbekistan: Solving a Dating Controversy	B-2
14:05		Watanabe	Chemical Process to Separate Magnetite Particles in Pottery Sample for ESR Dating	B-3
14:25		Blackwell	ESR Analyses for the Paleolithic Site at Attirampakkam, India: Clues to Complex U Uptake and Paleoenvironmental Change	B-4

14:40		Usami	Decay of the ESR signals in quartz by the high speed friction experiments: Basis for dating of fault movements	B-5
14:55		Baffa	On the Influence of Carbonate Calcite Contamination on ESR Dating	B-6
15:15	**Session C: Intercomparison Studies** **Chair: V. Chumak**	Toyoda	Interlaboratory comparison on Tooth Enamel Dosimetry on Semipalatinsk Region: Part 1, General View	C-1
15:30		Ivannikov	Interlaboratory comparison on Tooth Enamel Dosimetry on Semipalatinsk Region: Part 2, Effect of Spectra Processing Procedure	C-2
15:45		Lloyd	The Assimilation of Cytogenetic, ESR and Biochemical Assays For Highly Exposed Victims	C-3
16:00	**Afternoon Break**			
16:15	**Session D: Fundamental Studies on Tooth Enamel** **Chair: Bonnie Blackwell**	Fattibene	Dosimetric properties of the incisor teeth. Part 1: Influence of sample mass on dose estimate	D-1
16:30		Nakamura	A method to differentiate between the levels of ESR signals induced by sunlight and by ionizing radiation in teeth from atomic bomb survivors	D-2
16:45		Tikunov	Lower bound of enamel radiation sensitivity to neutrons	D-3
17:00		Khailov	Enhancement of the EPR sensitivity of tooth enamel to neutrons at irradiation in the human head phantom	D-4
17:15		Rudko	Temperature-stimulated transformation of radiation induced CO_2 in tooth enamel plates	D-5
17:30		Kirillov	Analysis of EPR tooth enamel spectra exposed to combined radiation and mechanical effects	D-6
17:45	**Close Conference**			

Tuesday, 11 July 2006

	Session	Speaker	Title	Page
8:30 8:45	**Radiation Epidemiology/BIER-VII** **Chair: David Schauer**	Mabuchi (Plenary Speaker)	Radiation dose and epidemiological risk estimation in atomic bomb survivors	S-3
9:00 9:15		Lloyd (Plenary Speaker)	A focus on cytogenetic dosimetry	S-4
9:30 9:45	**Session E: Biodosimetric Methods** **Chair: Dr. Wilkinson**	Voisin (Invited Speaker)	Needs for a standardisation of biological dosimetry by cytogenetics in expertise situations and population triage	E-1

10:00		Bhatti	International Study of Translocations in Control Populations	E-2
10:15		Tawn	FISH Chromosome Analysis of Sellafield Radiation Workers with Internal Deposits of Plutonium	E-3
10:30				
10:45	**Morning Break**			
11:15		Livingston	An Automated Method to Quantify Radiation Damage in Human Blood Cells	E-5
11:30		Prasanna	Laboratory automation and information management for cytogenetic biodosimetry	E-6
11:45		Miller	Depleted Uranium Mutagenesicity and Deletion Pattern Analysis at the Hypoxanthine Phosphoribosyl Transferase (HPRT) locus: Method to Discriminate Depleted Uranium Exposure from Other Genotoxic Poisions	E-7
12:00		Poster Session 2	Retrospective EPR: Ciesielski, Kuterbekov, Trompier	P-25..29
12:15			Semipalatinsk subgroup: Fattibene, Pivarov (2), Sholom, Stepanenko, Zhumadilov	P-30..35
12:30	**Lunch & Posters**		Biodosimetry poster abstracts: Lindholm, Kodama, Roy, Stricklin, Wilkins	P-36..40
12:45			Acute Dosimetry: Trompier, Grace	P-42..43
13:00				
13:15				
13:30	**Session F: EPR Methods**	Wieser (Plenary Speaker)	Inter-laboratory comparison of EPR dosimetry with tooth enamel in the SOUL project	F-1
13:45	**Chair: Paola Fattibene**			
14:00		Rugge	Dosimetry based on environmental objects	F-2
14:15		Pass	Radiation Exposure Measurements for Military Participants in US Nuclear Weapons Tests Using EPR in Dental Enamel	F-3
14:30		Chumak	Applicability of EPR dosimetry with teeth to dosimetric support of epidemiological studies: practical aspects	F-4
14:45		Stepanenko	Semipalatinsk Overview	F-5
15:00				
15:15		Hayes	Radiological Emergency Response Dosimetry in the United States	F-6

15:30	**Afternoon Break**	
15:45 16:00 16:15 16:30 16:45 17:00 17:15	**Consensus Session #1 - Retrospective Dosimetry**	Dr. Steve Simon (Chair) Dr. Andre Bouville (Deputy Chair)
17:30	**Close Conference**	

Wednesday, 12 July 2006

	Session	Speaker	Title of Talk	Page
8:30 8:45	**Acute Dosimetry** **Chair: C. Norman Coleman**	Gonzales (Plenary Speaker)	Potential Scenarios of Terrorist Attacks and Radiation Accidents: The Need for Retrospective Biological Dosimetry of Acute Radiation Overexposures	S-5
9:00 9:15		Jarrett (Plenary Speaker)	Medical Treatment of Radiation Injuries—Current U.S. Status.	S-6
9:30 9:45	**Session G: EPR Applications** **Co-Chairs: Anne Skinner and Nori Nakamura**	Swartz (Plenary Speaker)	Overview of *In Vivo* EPR, Including Use in Human Subjects	G-1
10:00		Trompier	Electron Paramagnetic Resonance radiation dosimetry with fingernails	G-2
10:15		Williams	Overview of the L-band EPR dosimetry results in vitro. Optimization of conditions of data collection and development of software for data collection and analysis in emergency in vivo dosimeters.	G-3
10:30 10:45	**Morning Break / Group Picture**			
11:00		Sucheta	Statistical Methods for Dose Reconstruction Using in Vivo EPR Tooth Dosimetry	G-4
11:15		Bhattacharyya	Implementing EPR Dosimetry for Life Threatening Incidents: Factors Beyond Technical Performance	G-5
11:30		Romanyukha	Mechanically-induced signal in fingernails as a confounding factor for EPR dosimetry	G-6
11:45		Carr	EPR Dosimetry for Radiation Emergency in WHO-REMPAN Collaborating Centers and Liaison Institutions	G-7

12:00 12:15 12:30 12:45	**Lunch**			
13:00	**Session H: Biodosimetric Applications** **Chair: David Lloyd**	Yoshida	Chromosome Network for Biodosimetry in Japan	H-1
13:15		Wilkinson	Canadian Biodosimetry Capacity	H-2
13:30		Stricklin	Biodosimetry Inter-comparison: FOI and DRDC Ottawa	H-3
13:45		Albanese	Building Connecticut's Clinical Laboratory Surge Capacity to Mitigate the Health Consequences of Radiological and Nuclear Disasters	H-4
14:00		Roy	Optimization of Cytogenetic Procedures for Population Triage in Case of Radiological Emergency	H-5
14:15		Grace	Quantitative Expression of p53 and STAT3 Dependent Genes in Relevant Models for Biodosimetry Applications	H-6
14:30		Ossetrova	The Use of Disciminant Analysis for Evaluation of Early-Response Multiple Protein Biomarkers of Radiation Exposure Using Non-Human Primate 6-Gy Whole-Body Radiation Model	H-7
14:45		Blakely	Multiparameter and Integrated Biological Dosimetry - Protein Biomarkers and Biodosimetry Medical Recording Tools Supporting Radiation Casualty Incidents	H-8
15:00	**Afternoon Break**			
15:15 15:30 15:45 16:00 16:15 16:30	**Consensus Session #2 - Acute Biodosimetry**	Dr. Harold M. Swartz (Co-Chair) Dr. George A. Alexander (Co-Chair)		
16:45	**Depart for Conference Banquet**			

Thursday, 13 July 2006

	Session	Speaker	Title	Page
8:30	**Session I: Fundamental Studies on EPR in Alanine**	Dolo	Improvement in the fabrication process of alanine pellet: influence on the angular response and fading	I-1
8:45	**Chair: Albrecht Wieser**	Ciesielski	Dose dependence of sensitivity of alanine radicals to visible light	I-2
9:00		Garcia	Study of various radicals proportions in simulated alanine spectra	I-3
9:15		Baffa	Small radiation fields dosimetry with L-alanine and 2 metilalanine K-Band EPR miniature dosimeters	I-4
9:45	**Session J: New Materials in EPR and Biological Dosimetry**	Marrale	Improvement of sensitivity in ESR γ-dosimetry by gadolinium addition	J-2
10:00		Ferraz	EPR and TL dating of Dioptase (Chrysocolla) crystal	J-3
10:15	**Chair: Steve McKeever**	Hole	Lithium formate as a low-dose EPR radiation dosimeter	J-4
10:30	**Morning Break**			
10:45	**Session K: Emerging Technologies**	Yukihara	The potential of Optically Stimulated Luminescence (OSL) of dental enamel for retrospective assessment of radiation exposure	K-1
11:00	**Chair: Sandra Eaton**	Goans	Ultrasonic Analysis of Acute Thermal and Radiation Injury	K-2
11:15		Kuppusamy	New Probes for Environmental and Clinical Applications of EPR Spectroscopy	K-3
11:45		Turteltaub	Technology Assessment and Roadmap for Emergency Radiation Dose Assessment	K-5
12:00	**Conference Wrap-Up**			
12:15				

12:30	**Satellite Session: Kazakhstan Nuclear Sites** **Co-Chairs: M. Hoshi and V.F. Stepanenko**	Stepanenko	The 1st Nuclear Test in the Former USSR of 29 August, 1949: Comparison of Individual Dose Estimates by ESR Retrospective Dosimetry with Calculation and Luminescence Retrospective Dosimetry Data for Dolon' Village, Kazakhstan	P-34
12:45		Zhumadilov	Results of tooth enamel EPR dosimetry for population living in the vicinity of the Semipalatinsk Nuclear Test Site	P-35
13:00		Sholom	EPR tooth dosimetry of Semipalatinsk area inhabitants	P-33
13:15		Hoshi	Interlaboratory comparison on Tooth Enamel Dosimetry on Semipalatinsk Region: Part 1, General View	C-1
13:30		Kuterbekov	EPR Investigation of Radiation Situation in Vicinity of Tailing Pool Koshkar-Ata.	P-27
13:45		Pivovarov	EPR and γ-spectrometeric researches of bottom sediments and soils in Syr-Darya uranium-ore province	P-31
14:00		Pivovarov	Exposure Subpopulations and Characteristics of the Individual Dose Distribution Among Inhabitants of the Semipalatinsk Region	P-32
14:15		Wieser	Inter-laboratory comparison of EPR dosimetry with tooth enamel in the SOUL project	F-1
14:30		Toyoda	ESR Dosimetry and 90Sr Distribution, Detected by Imaging Plates, of Cow Teeth from Southern Urals	P-9
14:45	**Close Satellite Session**			

Overview of Biodosimetry Projects in the NIH Radiation/Nuclear Countermeasures Program

Narayani Ramakrishnan, Andrea DiCarlo, Bert Maidment, Richard Hatchett

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Very few medical products exist to counter the variety of acute and long-term injuries that can result from nuclear or radiological attacks. In addition, there is currently no high throughput post-exposure method available to measure the radiation dose received by individuals. Because triage and medical treatment decisions depend on understanding the dose an individual receives, the development of biodosimetry devices that can rapidly and accurately distinguish individuals who need therapy from those who do not is critically important.

The Centers for Medical Countermeasures against Radiation (CMCRs) are multidisciplinary extramural research centers supported by NIAID and comprised of academic, commercial and government laboratories. The CMCRs support basic, translational, and applied research leading to new treatments for radiation injury. Biodosimetry projects in the CMCRs include development of 1) high-throughput, minimally-invasive, robotics-controlled automated image acquisition systems that will analyze micronuclei and H2AX –loci from lymphocytes, exfoliated buccal cells, and urinary bladder cells; 2) a biodosimetry tool with a fully integrated biochip and an integrated micro/nanofluidic cartridge that can perform whole-blood microarrays for radiation-injury-specific gene expression signatures; 3) a portable biodosimeter based on radiation-induced metabolomics expression signatures; 4) hand-held lateral flow diagnostics based on radiation-induced protein biomarkers; 4) flow-cytometry based high-throughput bone marrow biodosimetry assay that can measure cytogenetic damage to hematopoietic cells in bone marrow; 5) techniques for assessing skin exposure by measuring DNA damage in skin cells; 6) a hand-held EPR dosimeter using nails, hair and tooth chips; and 7) a biodosimeter based on optically stimulated bioluminescence. The centers will conduct basic research to identify the biomarkers of radiation damage as well as develop state of the art devices and translate the basic knowledge into products for use in the field.

In addition, NIAID has established interagency agreements with the Armed Forces Radiobiology Research Institute (AFRRI) and the National Cancer Institute (NCI). Under this agreement, AFRRI will develop standard operating procedures for and conduct an interlaboratory comparison of the dicentric cytogenetic assay. AFRRI will also develop automated techniques to facilitate initial processing of 500 or more samples in a 7-day interval. NCI will conduct epidemiological studies to develop computer modeling for dose estimation.

ESR – Global Player in Dosimetry

D.F. Regulla

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Sixty-five years of research have substantiated ESR spectrometry as reliably encompassing major key sector areas in quantifying radiation, e.g. transfer and secondary standard dosimetry, cancer treatment and radiation processing, dosimetry control of food irradiation and retrospective dosimetry of accidentally exposed humans or animals using their tooth tissues. Quantifiable ESR signals have also been obtained from bricks, wood, soil and other environmental materials, after radiation accidents. ESR based individual dosimetry was recognized early on as an excellent tool for studying potential long-term health effects as a precursor for epidemiological risk estimates concerning, e.g., Chernobyl population and liquidators, South Ural population and Mayak nuclear workers, Hiroshima A-bomb survivors, residents near the Semipalatinsk nuclear test site and some other cases of radiation accidents, e.g. Toka-mura, Japan and Goiania, Brasil. Detailed methods are now summarized in IAEA-TECDOC-1331 and ICRU Report 68. In addition ESR has provided a successful tool for identifying irradiated food and for archeological and geological dating of hominid sites from the paleolithic through to more recent times. The potential and diversity of ESR physical and biophysical dosimetry appears unrivalled, world-wide, by any of the other established traditional dosimetry methods. While ESR dosimetry methods are already used routinely for invasive accident dose reconstructions, there is a strong need for evaluating its metrological potential as a non-invasive biophysical dosimetry with human tissues *in situ* and thereby using ESR dosimetry in situations of life threatening incidents for individuals who do not wear special physical dosimeters.

Recognizing the global impact of security concerns, a fast-processing biophysical dosimetry strategy and standardization deserves urgent attention and innovative methods development. *In vivo* dosimetry measurements on teeth and bones for rapid response triage, in order to identify casualties who could develop acute clinical effects, is the subject of a novel ESR retrospective dosimetry method presently under fast development using L-band (1.2 GHz). Any new ESR technology should complement biological dosimetry by cytogenetics and hopefully plug the time gap caused by the need for processing of blood and cell scoring.

Apart from some mutual agreements in the world on assistance networking using cytogenetics, no international standard currently exists for large-scale emergency measurements. Yet, unexpected disasters ranging from potential nuclear accidents to radiological terrorism underscore the importance of metrological consensus, e.g., on biological and biophysical dosimetry, and international coordination in emergency cases. Radiological preparedness, anticipating situations of population triage, is a vital part of a country's infrastructure.

Electron Spin Relaxation Times of Radiation-Induced Defects in Teeth.

Hideo Sato, Sandra S. Eaton, and Gareth R. Eaton

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Quantitative measurements of the electron paramagnetic resonance (EPR) spectra of the defect centers induced in teeth by radiation need to consider microwave power saturation of the EPR signals. To guide EPR dosimetry methodology development, direct measurements of electron spin relaxation times are needed. Measurements of T_1 by pulsed saturation recovery and inversion recovery, and of T_2 by pulsed electron spin echo methods will be reported. Samples include several irradiated tooth fragments and a sample of synthetic hydroxyapatite.

Accuracy Considerations in EPR Dosimetry
Part 2—Regression-Related Aspects

Vitaly Nagy

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Even with a given dosimetry system and instrumentation, accuracy and precision of measured doses may be significantly different depending on the technique used to relate measured signals with absorbed doses.

An EPR dosimetrist has significant latitude in selecting specific calibration technique (e.g., back-extrapolation vs. a calibration curve), width of the calibration range, number of standard doses in the calibration range or number of added doses, specific values of the calibration doses, number of the replicate standard samples irradiated to the same dose, number of the replicate measurements of the signal of each sample, specific function to fit the data points, type of regression (weighted vs. unweighted and the method of weighing in the latter case; classic vs. "orthogonal"), algorithm for processing data of "orthogonal" regression, whether or not to force the line to go through the origin of the coordinates, number of replicate measurements of the signal of each unknown sample, and some others.

All these factors affect the accuracy and precision of the final result, in many cases very significantly. It appears that this aspect of EPR dosimetry has had much less attention than many other areas, and EPR dosimetrists usually employ the most straightforward regression techniques and the simplest schemes without an attempt at optimization. Even worse, the simplest techniques often are incorrectly used in situations where the most basic assumptions of these techniques are not valid.

Fortunately, a great deal of work has been done in an attempt to clarify these important issues in the other sciences that use calibrations, primarily analytical chemistry. Closed analytical expressions have been derived for simple cases, which make it possible to analyze the dependencies and get valuable guidance for optimization. In more complicated cases, a variety of numeric methods can be used for the same purpose. EPR dosimetrists can and should take full advantage of these possibilities by adjusting and applying the available techniques to their specific problems.

In the previous lecture of this series in Obninsk, only passing notes could be given on these issues. In the present lecture, a review of the achievements, possibilities, and specific recommendations will be given based on the extensive literature data and our own results.

Rapid-Scan EPR: A New Magnetic Resonance Method with Potential Application to Dosimetry.

Amarjot Dhami, George A. Rinard, Richard W. Quine, Mark Tseytlin, Sandra S. Eaton, and Gareth R. Eaton

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Most EPR dosimetry has been performed using continuous wave (CW) EPR. Some relaxation times of radiation defects have been measured using pulsed EPR. We are developing a new EPR method, called rapid scan EPR, in which the magnetic field is scanned rapidly relative to relaxation times of the sample. The effect of rapid magnetic field scans on the signal and methods of deconvolving these effects will be described. Rapid scan EPR yields the absorption curve instead of the derivative, which has important advantages for EPR imaging, since the signal amplitude decreases linearly with applied magnetic field gradient, rather than quadratically as is the case for normal derivative spectra.

Profile likelihood for dose estimation in radiation dosimetry studies

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A common problem in dosimetry is to estimate the dose of irradiation received by an exposed individual using a dose-response curve. A number of statistical methods have been designed for that particular purpose. Many of them consist of inverting a dose-response relationship, which has been preliminary estimated during a calibration study. While such a general approach has proven to be particularly useful for a wide range of applications, its limitations can also be attained in many practical situations. An interesting example, often encountered in practice, is when multiple measurements are collected on the exposed individual. A natural question in this context is how to combine these measurements together to achieve good point and confidence estimates for the dose. We suggest making use of the likelihood theory and profile likelihood in such cases. In this talk, we will describe this approach, and present an application to the MN-retyculocyte assay to demonstrate the power of the proposed method.

Comparison of ED values from fossil tooth enamel samples from Pleistocene period using various weighting protocols

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An accurate determination of the Equivalent Dose (ED) is critical for dating archaeological and geological samples by ESR method. Fort tooth enamel, ED is determined by the additive method which allows to describe by gamma irradiations the evolution of the ESR intensity I (reliable to the filled traps) versus the dose. The ED is obtained by extrapolation using an exponential fitting from the experimental growth curve.

The results may vary significantly depending the curve shapes and the experimental data and protocols. This work focus on ED determination and its uncertainty. Thus, different protocols: without weighting, weighting using the natural point, 1/I (I: ESR Intensity) weighting, $1/I^2$ weighting and $1/\sigma^2$ (σ: standard deviation of I) have been checked. The studied known age samples have been chosen in order to cover all the Pleistocene period (20 ka to 1.8 Ma) with different ratios I highest dose/I unirradiated. The ED values and their uncertainties obtained with the different protocols are discussed according to their compatibilities. The ED influence on the age and its uncertainty is analyzed depending the age accuracy.

Coupled ^{230}Th/^{234}U-ESR Analyses for Corals: A New Method to Assess Sealevel Change

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Abstract

Although coupled ^{230}Th/^{234}U-ESR dating has become routine for dating teeth, it has never been used to date corals. Both ESR and ^{230}Th/^{234}U can date coralline aragonite ranging in age from ~ 1 to 400 ka. In ESR, the age depends on the time-averaged cosmic dose rate, $\overline{D}_{\cos}(t)$, assumed, but the ^{230}Th/^{234}U dates do not depend on $\overline{D}_{\cos}(t)$. Because $\overline{D}_{\cos}(t)$ experienced by corals has varied with time, particularly in response to changing water depths due to subsidence, tectonic movement, and sea level change, determining a unique ESR age can require assumptions about $\overline{D}_{\cos}(t)$. By coupling the two methods, one can determine the age and $\overline{D}_{\cos}(t)$ simultaneously. For stable platforms, $\overline{D}_{\cos}(t)$ depends on the water depth and thickness of coral overlying the dating sample. Hence, $\overline{D}_{\cos}(t)$ from the coupled analysis can be used to estimate a water depth profile that can be compared to $\overline{D}_{\cos}(t)$ values estimated from model sea level curves. If the two $\overline{D}_{\cos}(t)$ values agree well, this provides independent validation for the sea level model.

For six corals from the Florida platform, their coupled $\overline{D}_{\cos}(t)$ values and ages agreed well with the sea level curve from Waelbroeck *et al.* (2002). This study demonstrates a general applicability of the method. More precise measurements for water depth and coral cover thickness would improve the precision in fully testing the sea level curves. Where one large coral head can be sampled at several points, or a reef can be sampled over a transect, the precision for the predicted $\overline{D}_{\cos}(t)$ would increase dramatically for testing sea level curves.

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Absorbed doses in tooth enamel due to external and internal irradiation of human body by ^{134}Cs and ^{137}Cs isotopes

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As result of the Chernobyl accident in 1986 irradiation of population is mostly caused by contamination of territories by radioactive isotopes of ^{134}Cs and ^{137}Cs. External irradiation is caused by gamma radiation from the cesium isotopes in the environment. Internal irradiation is caused by the isotopes incorporated in the human body as result of inhalation, consumption of contaminated food, water, etc. The problem of dose reconstruction for such radiation contamination may be resolved by the method of human tooth enamel EPR spectroscopy (EPR-dosimetry). The aim of the present work is estimation of contribution of internal and external radiation from ^{134}Cs and ^{137}Cs to dose absorbed in teeth enamel, which is necessary for analysis of the EPR dosimetry results.

Absorbed dose in the enamel and whole body dose were calculated using Monte Carlo (MCNP-4B code) simulation of ionizing particles transport for external and internal radiation from ^{134}Cs and ^{137}Cs isotopes. Calculations were performed for realistic adult male human body phantom with added dental part. Both gamma and beta radiation components of cesium decay were taken into account at calculation of internal dose caused by incorporated isotopes uniformly distributed in soft tissues of the phantom. External dose was calculated taking into account gamma radiation from isotopes distributed on the flat disk simulating contaminated earth surface. Ratio of dose absorbed in enamel to whole body dose caused by internal irradiation was found to be of 0.46+/-0.04 and 0.35/+/-0.04 for ^{134}Cs and ^{137}Cs isotopes respectively. For external radiation, that ratio was found to be of 0.96+/-0.04 for both isotopes.

A portable accident dosimeter using tooth enamel

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Due to the increasing use of ionising radiation, for example in food, nuclear power, quality assurance and armaments industries, is the possibility of radiation accidents more likely. In addition, the risk of terrorist attacks using radio isotopes is becoming ever more menacing. The EPR technique is well known for retrospective dosimetry using tooth enamel. However, despite intensive investigations, no adequate in-vivo solution yet exists.

Microwave radiation coupling using a loop resonator together with a permanent magnet field now appears to offer an optimal solution to in-vivo dosimeter. The measurement of tooth enamel radicals directly in the mouth using new L-band (in preference to X band) microwave detection methods are the main contributions of this work. The goal is the development of a reduced weight and volume EPR spectrometer suitable for use in field studies. For the determination of accuracy, repeatability and detection limits future investigations has to be done.

Dosimetric properties of the incisor teeth. Part 2: Comparison of EPR dose response of enamel from incisors and molars

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Electron paramagnetic resonance (EPR) spectroscopy of tooth enamel from molars has achieved a leading position for the reconstruction of individual doses. A major application of the method is the retrospective assessment of external doses for improvement and validation of independent dose estimates for use in radio-epidemiological studies. In most cases the cohorts under study were exposed several decades ago and the members are of high age nowadays. For those cohorts incisors are more frequently available than molars. However, at present only molars are recommended for use in dose reconstruction due to possible effects of solar UV to incisors. In order to improve applicability of EPR measurements of tooth enamel a series of investigations on the dosimetric properties of incisors was started to evaluate possibilities of their use in dose reconstruction.

The part 2 of the investigation series is aimed at evaluating differences in radiation sensitivity of enamel from the lingual and buccal part of incisors and the comparison with the sensitivity obtained from enamel of molars. The part 1 of the investigations is dedicated to evaluate the influence of sample mass on the quality of EPR measurements.

The investigations were done with tooth samples of donors from uncontaminated areas in Russia and Egypt. EPR-signal-to-absorbed-dose response curves were evaluated in the dose range up to 1.5 Gy with aliquots of 100 mg of pooled enamel powder from buccal and lingual parts of incisors, and of molars. The current results give no evidence for differences in radiation sensitivity of enamel from different parts of incisors and from molars.

γ- and UV-induced CO_2^- radicals in tooth enamel

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γ-irradiation of the tooth enamel causes EPR signal that is used in EPR dosimetry and EPR dating. Two types of defects contribute to the spectrum, namely, the oriented and disordered CO_2^- radicals. Recently it was found that the same radicals can be produced by UV-light exposure. Since the EPR parameters of the radicals do not depend on the energy of the incident quanta the problem is to distinguish between the doses from different radiation sources.

The present report deals with the comparative study of the EPR spectra caused by γ- and UV- irradiation of the enamel. The samples were the clinically sound enamel plates cut of the intact tooth with the help of dental instruments and cleaned with nonabrasive polishing paste. One plate was exposed to ^{60}Co γ-rays, another – to UV light. The irradiation was carried out in air at room temperature. The irradiation doses were adjusted so that the intensities of the EPR signals of both samples were approximately equal. The exposure was about 100 hours for the UV irradiation and the γ-rays absorbed dose was about 4 kGy. EPR measurements were carried out using X-band spectrometer at room temperature. The magnetic field was modulated at 100 kHz with peak-to-peak amplitude 0.2 mT.

The investigations of radiation-induced EPR spectra at different orientations of magnetic field showed that angular changes of the EPR lineshape are more pronounced for the UV-irradiated plate as compared with the γ-irradiated one. These changes of EPR spectra are caused by the oriented CO_2^- radicals while the disordered radicals form the angular-independent signal. Thus, the results obtained evidence that the UV-exposure produces larger quantity of oriented radicals than γ-irradiation at approximately equal total amount of CO_2^- radicals.

In order to determine the oriented to disordered species ratio we fit the simulated EPR spectra to experimental ones for different orientations of external magnetic field. To simulate the disordered CO_2^- EPR lineshape we used the parameters: $g_x = 2.0030$, $g_y = 2.0015$ and $g_z = 1.9975$. Experimental EPR spectrum of the annealed enamel plate was used to fit the contribution of oriented species. The analysis showed that the contribution of the oriented CO_2^- radicals in UV-exposed sample is 30-37% while in γ-irradiated sample is 16-20%. The possibility to distinguish the doses from different radiation sources is discussed.

Absorbed dose profiles in teeth studied by EPR: energy dependence

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In the case of emergency dose assessment due to a Chernobyl-like accident, one of most uncertain factors influencing on accuracy and reliability of EPR dose reconstruction technique with teeth is energy of the gamma radiation. Frequently the spectrum of the gamma-field is unknown and the special assumptions are required in order to convert accurately the EPR-reconstructed tooth enamel doses into some generally accepted reference values and/or doses for vital human organs and tissues.

The information needed to assess the energy of accidental exposure may be obtained from measurements of dose profiles in teeth. It is known that gamma radiation attenuates in the range of several percent (for radiation energy few hundreds keV and higher) to factors of ten (for low energy) when it passes through a dentine/enamel layer with a thickness of 8-10 mm (typical size of a molar). This fact may be exploited to develop a technique to estimate the effective radiation energy using measurements of dose profiles in teeth. It is appropriate to mention here that in addition to the energy of radiation, there are some factors which may influence on dose profile shape in teeth. One major factor is the geometry of the *in vivo* tooth exposure (e.g. the tooth may be exposed from different directions) as well as the scattered radiation, whose contribution to dose will vary for different layers of a tooth. Obviously these and other factors may significantly complicate the interpretation of dose profiles in teeth.

The dose profiles in teeth were experimentally and theoretically investigated in the present study. Teeth were positioned inside a head phantom and exposed to different energies of gamma- and X-ray beams in the range from 38 keV (heavy-filtrated X-ray source H50) to 1.25 MeV (gamma-source Co-60). Four molars were exposed at each energy, then sliced into 2.5 mm layers and measured by EPR. Dose profiles were plotted using mean values and standard deviations for the same depth layers. The resultant dose profiles were analyzed in different ways and two important effects were found: an absence of dose profiles for energies higher than 120 keV and, for low-energy heavy-filtrated beams, dose profiles that were attenuated by only a factor of two (approximately) between first and last tooth layers. The experimental profiles obtained from these data were used as references for corresponding Monte-Carlo simulations.

Geological Dating of Zircon using ESR method

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Zircon, $ZrSiO_4$, can be used for geological dating, and here we are concerned with the age of natural zircon samples collected from uranium mine located at Poço de Caldas, Brazil. These samples, called caldasito, are opaque with 2,5 to 3 cm in diameter and 3 to 4 cm in length. Assuming that UV light affects only a surface layer not larger than 1 mm deep, the internal part of each sample was selected, crushed and sieved appropriately for ESR measurements. Due to relative high Fe content, a broad signal, about 1000 G wide, was observed at the 3000 - 4000 G region. However, in an enlarged region from 3465 - 3485 G region a g = 2.000 signal that grew with increasing radiation dose was observed. From a plot of this ESR signal intensity vs. radiation dose up to 5 kGy an accumulated dose of about 890 Gy was obtained.

The ESR measurements for the external parts of the samples (a skin of about 2 - 3 mm) were carried out. No significant difference between the accumulated dose value for the internal part and that for the external part was found.

The ESR signal at g=2.000 showed strong effects on exposure to UV. In about 100 min the signal intensity reduces to half the initial value. Note, however, that no matter how long the UV exposure, the intensity did not reduce beyond a residual value about half the initial value.

The result of the present work indicates that, unless one collected a very thin surface material no difference between “surface and internal” materials can be detected and, furthermore, the internal part of the samples, here used, can give the age of zircon as found in mine.

ICP-MS analyzes for the samples yielded 445ppm uranium and 165ppm thorium. From these values obtained we could calculate an annual dose rate.

ESR Dosimetry and ^{90}Sr Distribution, Detected by Imaging Plates, of Cow Teeth from Southern Urals

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Tooth enamel is an excellent material for ESR (electron spin resonance) retrospective dosimetry. Examining the human teeth has the great advantage that each dose given to each individual could be determined. Unfortunately, human teeth are not always available. In such situations teeth from animals could be used for retrospective dosimetry.

As results in early period of the operation of Mayak, which is the first Soviet nuclear weapon plant, substantial amount of radioactive wastes were dumped into Techa River. Also in 1957, one of the radioactive waste facilities was exploded because of the failure of the cooling system, which caused the spread of radioactive wastes, mainly composed of beta emitters, to the vicinities. In the present study, we examined the cow teeth taken from the farms nearby in South Ural region where the level of the contamination is now below 3.7×10^{10} Bq/km^2.

The imaging plates sensitive to beta rays were employed to observe the spatial ^{90}Sr distribution in the cow teeth It was found that ^{90}Sr is concentrated in dentin part of the teeth up to 10 Bq/g. The average concentrations in teeth tend to be higher in the region of higher contamination level, but not all. Some data imply the correlation with the contamination level of food for cows. The radiation doses accumulated in the teeth were obtained by ESR (electron spin resonance) method. Five samples showed noticeable radiation dose up to 200 mGy. Four of them showed also high ^{90}Sr concentration in the teeth whereas the other one did not.

EPR measurement and Monte Carlo calculation of dose conversion coefficients for deciduous teeth

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The greatest hazards from radiation incidents in the proximal populations will be to children since they have a higher radiation risk per unit dose than adults. Therefore, the provision of a methodology to reconstruct individual doses for children on the basis of EPR measurements with enamel from deciduous teeth is of great importance. The use of deciduous teeth in EPR dose reconstruction has the unique potential to assess individual doses that were accumulated in the early childhood in the age up to 12 years. It was found previously that deciduous incisors are only of limited use in dose reconstruction but deciduous molars have high radiation sensitivity and are well suited for reconstruction of low absorbed doses in tooth enamel.

The assessment of individual doses for children by EPR measurement of tooth enamel requires conversion of the absorbed dose-in-enamel to absorbed dose-in-free-air for calculating radiation risk relevant quantities like organ doses or whole body dose. Dose conversion coefficients for external exposure depending on tooth position, photon energy in the range from 10 keV to 10 MeV and geometry of the radiation field had been calculated by Monte Carlo simulation using a mathematical model for deciduous teeth and a modified MIRD-type mathematical phantom of a 5-year-old child.

In the current study, deciduous molars were irradiated inside of a simplified phantom representing a child's cheek for verification of the tooth model by comparing dose conversion coefficients obtained from EPR measurement and Monte Carlo calculation. Irradiations were performed with photon beams of X-rays of 63 keV equivalent energy and ^{60}Co gamma rays (1.25 Mev). Calculated and measured dose conversion coefficients for the plate phantom are presented and compared with calculated results for the mathematical child phantom.

Irradiation dose control of chicken meat processing with Alanine/ESR dosimetric system

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Irradiation of foodstuff is a well known technique to preserve food. In our country spices are already irradiated for sanitary and preservation reasons. Chicken meat is an important commodity; Brazil is the second world producer and the largest world exporter. The shelf life of chicken meat is limited by the presence of microorganisms and enzyme activity and together with other preservation techniques irradiation seems to be an attractive option. In this study the dose delivered to frozen chicken cuts were measured and compared with the prescribed value. Chicken breast cuts were analyzed during 39 days for their microbiological activity, chemical and organoleptic properties.

Cylindrical dosimeters were prepared using the weight composition of 80% of DL and L-alanine (Sigma Co), used without any further treatment except drying, and 20 % of paraffin. The dosimeters having 4.7 mm diameter and 12 mm length were inserted in a build-up cap. Dosimeters were placed inside cardboard boxes containing frozen chicken breast cuts, packed in styrofoam trays wrapped with plastic film. The boxes were irradiated in an industrial ^{60}Co irradiator (Nordion JS 7500) with a dose rate of 4kGy/hour. First derivative ESR signals were obtained in a VARIAN E-4 spectrometer operating at X-band ($\nu \approx 9$ GHz) and equipped with a rectangular cavity (TE-102, model E-231). The cavity was constantly purged with dry nitrogen and modulated at 100 KHz with 0.5 mT peak to peak. A calibration curve was made for a few dosimeters from the same batch and used to obtain the dose from the ESR signal intensity.

A batch of six boxes was irradiated at each experiment with prescribed doses of 1.5, 3.0 and 7.0 kGy. The dosimeters were placed in different positions in the cardboard box. The measured doses had a standard deviation from 8% to 13%. Comparing the possible dose variation with the microbiological, chemical and organoleptical properties it is possible to prescribe a dose that would allow preservation without compromising the food quality.

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Dose Fractionation Effect in Alanine/EPR Dosimetry

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Many dosimetry methods based on chemical reactions suffer a so-called "dose fractionation effect," which means that a dosimeter response depends not only on the dose itself, but also on how this dose was delivered (uninterruptedly or in parts separated by breaks). This is a consequence of the fact that chemical reactions producing the response have a finite rate, and the regimen of the influx of new radiation-induced radicals into the system may significantly affect the kinetics or even the mechanisms of the prevailing reactions. In particularly unfavorable cases, such as with some radiochromic films, the dose fractionation effect can reach several tens of percent and requires significant efforts to correct for the resulting systematic errors.

It is widely believed that the alanine/EPR dosimetry system is free of this weakness. However, this is inconsistent with the ten-year-old observation that alanine signal amplitude changes in a complex way after irradiation, which depends on the absorbed dose (Nagy and Desrosiers, Appl. Radiat. Isot., 47, 789-793, 1996). These changes show that alanine signal is not just a result of a steady accumulation of the same instantly produced radicals but is based on chemical reactions of measurable rate. Therefore, it was reasonable to expect some fractionation effect.

A series of precise measurements were performed to check this hypothesis and to measure the magnitude of the possible effect. As the effect was expected to be small, a number of precautions were necessary to increase the precision of the experiment as much as possible; they will be described in detail.

We have found that a dose fractionation effect does exist in alanine dosimetry: an interrupted irradiation produces a smaller response than an uninterrupted one. The magnitude of the effect depends on the dose and the temporal pattern of the dose delivery. In the gray range, the difference is very small, but it can reach 0.5-1% in the most popular kilogray range. As the overall uncertainty of absolute dose measurements by the top laboratories is below 3% at 2σ, this is a significant value that needs to be taken into account in highly precise calibrations and intercomparisons.

Results of the experiments will be presented. Practical implications and possible solutions will be discussed.

Energy dependence and sensibility of different materials in EPR dosimetry for clinical x-ray beams

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EPR dosimetry has been studied for dating, retrospective dosimetry and emergency dosimetry. Recently, there has been an increasing interest in its use for radiation therapy dosimetry, and different kinds of materials have been used.

For radiation therapy and emergency dosimetry, it is preferable that the dosimeter possess the same properties as biological tissue, in order to avoid stopping power and mass energy-absorption coefficient corrections in the dose determination. For retrospective dosimetry and dating, the material must have no fading and a good stability. Some materials presenting these properties are DL-alanine, 2-methylalanine, sucrose, calcite and hydroxyapatite.

The energy dependence of these materials in EPR dosimetry in terms of dose-to-water was investigated for clinical 10 MV x-rays using ^{60}Co -rays as standard. Dose response curves were obtained, allowing a comparison between the sensitivities of the dosimeters. Small pellets of DL-alanine and 2-methylalanine (80% material and 20% paraffin) and gelatin capsules containing hydroxyapatite, sucrose and calcite, all in powder form, were irradiated with ^{60}Co and a LINAC (10 MV), receiving doses from 1Gy to 20 Gy. The EPR spectra of the irradiated materials were obtained with a Varian E-4 X-band spectrometer, using microwave power in a non-saturation range and high modulation amplitude of magnetic field in order to increase the dosimetric signal amplitude of the EPR spectra.

The dose response curves were a linear function and its slope was used to compare the calibration curves for the LINAC and the ^{60}Co. The ratio of the slopes were: $(96 \pm 5)\%$ for DL-alanine, $(92 \pm 5)\%$ 2-methylalanine, $(96 \pm 3)\%$ sucrose, $(107 \pm 14)\%$ hydroxyapatite and $(138 \pm 11)\%$ calcite. Sucrose was the most sensitive material, followed by 2-methylalnine, DL-alanine, hydroxyapatite and calcite.

Sucrose was an interesting material exhibiting a good sensitivity and energy response comparable with 2-methylalanine and DL-alanine. DL-alanine has been used in radiation therapy and 2-methylalanine has been suggested as a potential DL-alanine substitute. The higher energy dependence of hydroxyapatite and calcite might be caused due to he effective atomic number of these two materials, higher than others. Monte Carlo simulation and irradiation at other energies will be done in the future.

A study based on ESR, XRD and SEM of signal induced by gamma irradiation in eggshell

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ESR (electron spin resonance), XRD (x-ray diffraction) patterns and SEM (scanning electron microscopy) of irradiated calcium carbonate ($CaCO_3$) in from eggshell was investigated. The ionizing radiation produces an electron centre CO_3^{3-}, a hole centre CO_3^{-} and oxygen vacancy with an electron. The CO_2^{-} molecular ion also formed. The ESR centre with $g_{//}$ = 1.9970 and $g_{\perp}$ = 2.0012 was identified as the same found in hydroxyapatite. Additional ESR lines were detected, but they are less pronounced in intensity. The work aims to standardize the samples preparation method and the conditions of measured for practical application by the specialist in emergency dosimetry. In this regard, practical consideration of sample preparation conditions and properties such as grain size, ESR spectra, and the temperature dependence of the signal were studied in detail. The peak-to peak amplitude values of the derivative of absorption were recorded for relative dose measurements. At very low doses multiple sweeps were taken, resulting in a final composite spectrum. The spectrum of a non irradiated reference sample was subtracted. Dose response appears to be linear between 1 to 100 Gy. No dose rate dependence was observed. The morphology of the calcined eggshell presented extensive morphological change on the calcinations process. Careful analyses of the ESR spectra are presently in progress in order to undertaken and identify the radicals involved.

Effects of the doses and preheating on radiation induced signals in alkali feldspars

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It is known that heating can change the luminescence sensitivity of the crystals. A large number of luminescence studies carried out to analyze the origin of the sensitivity change, but few works have been done with ESR experiments. Nowadays the SAR protocols is largely applied in IRSL of feldspar for determining accrued radiation doses in unheated and heated samples, both for dating and accident dosimetry purposes, this protocol can corrected the luminescence sensitivity of the sample in regeneration method. In regeneration method using single aliquot, the same is submitted to several irradiations, optical stimulation and preheating cycles, these procedures can promote a change in luminescence sensitivity of the sample. Another example of sensitivity change occurs in the luminescence dating of materials previously submitted to heating, such as potteries, bricks and tiles. Therefore, a detailed study of effects of preheating in ESR signals can help in optical dating methodology research, in the present work ESR results of 4 alkaline feldspars will be presented and a correlation with emission spectra bands will be done.

ESR results have shown Fe^{3+}, Al-O^- - Al, Ti^{3+} centers in irradiated samples, with γ-radiation with a dose about 200Gy, we could note a decrease in intensity of the Fe^{3+} center in all the samples, but the Al-O^- - Al and Ti^{3+} centers kept almost constant. Annealing experiments were performed in samples with a previous dose of about 200Gy, the preheating temperatures used were 100, 200, 300 and 400°C, in this case it was observed an increase of the Al centers up to 300°C and after the signal intensity reduced. The Fe^{3+} center increased up to 200°C and became nearly constant for high temperatures.

Emission spectra have shown principal emission bands at around 360, 380, 450, 475, 500, 680, 725, 775 and 875nm. In the literature de Ce^{3+} is related to 355 and 490nm, Al center is associated with 450-480nm emissions, Ti^{4+} (Ti^{3+}= Ti^{4+} + e^-) with 460nm and Fe^{3+} with 700nm. According to ours results even low temperature preheating (200°C) can change the concentrations of Fe, Al and Ti centers in the sample, this decay could be related with low temperatures TL peaks emission mechanism in feldspar, and also can be associated with luminescence sensitivity changes, because all these centers are associated with one or more emission spectra bands.

EPR spectra of jade dosimetric samples

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Jade is the common denomination of two silicates: jadeite, $NaAl(Si_2O_6)$, and actinolite, $Ca_2(Mg,Fe)_5(Si_4O_{11})_2(OH)_2$, which belong respectively to the subclasses of pyroxenes and amphiboles. Green materials were acquired as jade with origin in New Zealand, Austria and USA. The dosimetric properties of these materials were already studied using the thermoluminescence technique, showing their potential use for high dose dosimetry[(1)]. In the present work they were studied using the EPR technique to investigate the potential applications in gamma radiation dosimetry in the range of 50Gy up to 10kGy. There are no evidences in the literature about jade applications in radiation dosimetry using the EPR technique; only crystalographical aspects of synthetic samples were compared to natural ones. All samples were initially cleaned, pulverized, and grain diameters between 0.074 and 0.177mm were obtained. The samples were thermally treated at 300°C during one hour in open atmosphere. The irradiation of the samples was made using a gamma-cell system (^{60}Co). EPR measurements were taken with a Bruker EMX spectometer. The EPR spectra of jade samples were obtained, and their main dosimetric properties as reproducibility, calibration curves and energy dependence were investigated.

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Resonator designs and concepts for enhancing *in vivo* dosimetry

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Sensitivity, reproducibility and performance of *in vivo* L-band EPR measurements are significantly affected by the characteristics of the resonators. It is widely accepted that each type of measurements requires improvement of a specific parameter of a resonator. Therefore EPR researchers persistently work on improvement of a resonator design for each kind of experiment (1-3).

Tooth dosimetry *in vivo* is relatively new area of *in vivo* EPR (4, 5). For this kind of measurements we developed resonators with loops that fit on the type of tooth being measured (molar or incisor) with a plastic protector to decrease the effect of moisture in the mouth on the Q-factor of the resonator. This development was performed using the extensive empirical knowledge of our group in resonator development.

We have developed a theoretical approach that can be used to defind optimal resonator parameters for EPR dosimetry more efficiently than purely empirical methods. This approach is based on the analysis of the resonator impedance using equivalent circuits including a lumped element model for the open circuited transmission lines (6). This requires: 1. creation of equivalent circuits of each part of the resonator and the whole resonator based on actual physical dimensions; 2. comparison and adjustment of the calculated resonant frequencies of the parts and a whole resonator and measured resonant frequencies; 3. optimization of the actual physical parameters of the resonator parts to adjust them to meet the required characteristics.

Preliminary experiments and calculations indicate that this approach could be useful for more investigating variations in the shape and size of a loop (a loop for several teeth, incisors etc.) and simultaneous variation of the length of the transmission lines and therefore facilitates the optimizing of L-band X-loop resonators for EPR dosimetry *in vivo*.

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Implementation of a 1.2-GHz tunable EPR resonator for tooth dosimetry

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We will report a development of a 1.2-GHz tunable EPR resonator that is used for *in vivo* tooth dosimetry. This resonator is based on an electronically tunable surface-coil resonator for EPR spectroscopy [1,2]. In addition to the usual technical considerations of continuous-wave (cw) EPR spectroscopy for animal experiments, specific additional requirements for *in vivo* tooth dosimetry in order to accommodate the need to place the resonator quickly, accurately, and comfortably within the mouth:

(1) Flexibility of the transmission lines
(2) Capability of having fast connection/disconnection of the resonator
(3) Easy and accurate physical alignment of the tip of the resonator with the tooth (teeth)

A possible solution for requirement #1 is to use flexible coaxial cables [3], instead of the semi-rigid cables that were used in our previous resonator. We also will investigate the use of SMB snap-on connectors for the transmission line that is connected to a surface coil. This feature could help to measure a large number of subjects in a limited time (requirement #2). For physical alignment of a coil on tooth (requirement #3), we will discuss possible approaches to properly locate the resonator on the teeth. Preliminary results of the prototype EPR resonator will be presented, in terms of meeting the above-mentioned requirements, as well as the sensitivity of the resonator.

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The Flat Magnet – A Portable Tool for EPR Tooth Dosimetry

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Today there is a credible threat of terrorist attack, including possible radiation exposure following a "dirty bomb" or even nuclear attack when many people may be exposed and proper medical treatment should be applied locally and rapidly. In such a scenario it is necessary to determine which people have received significant amounts of radiation exposure so that care can be applied efficiently, and the unexposed can be assured of their safety. Citizens do not usually wear portable dosimetry badges, so the determination of personal exposure is a challenge. However, such radiation generates radical centers in tooth enamel that are stable over very long durations (effectively permanent) that can be related to radiation exposure using EPR measurements. Conventional EPR spectrometers are heavy and therefore might be difficult to transport from site to site. A much smaller "flat" magnet" would be light and portable and could facilitate EPR measurements at the site of exposure.

Such an electromagnet has been designed and constructed by the Dartmouth EPR Center and Resonance Research, Inc. The flat magnet is a combination of coils on a single 26 cm diameter pole face which projects a magnetic field clear of the pole face with sufficient strength and homogeneity to make EPR tooth dosimetry measurements. The weight of the magnet with its base, power supply, and all necessary equipment is about 80 kg, which makes it readily transportable. It has wide-open access from its side where a human head can be positioned. During measurements, people can be seated in a comfortable and convenient position and measurements can be done very quickly without elaborate preparation.

A series of *in vitro* measurements proved that EPR signals from irradiated teeth can be recorded with this magnet. Isolated teeth with a range of applied doses were measured. The EPR signals were repeatable and consistent. The EPR signal is sensitive to the position of the tooth relative to the main magnetic field and modulation field and precise control is needed to acquire reproducible and reliable dose estimates. Devices for positioning the subject comfortably and accurately relative to the magnet are under development.

These results indicate that the flat magnet can be very useful for tooth dosimetry. The unique features of this magnet should enable EPR tooth dosimetry to be performed quickly and reliably in the field.

Possibilities for Rapid, Portable, Non-Invasive Dosimetry of Radiation Events Using Optically Stimulated Luminescence in Dental Enamel

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Currently, retrospective radiation dosimetry in humans lacks a technique that is sensitive, non-invasive, and portable. Without the ability to randomly sample and measure exposure in the general population, and to establish biodosimetric "truth," it is difficult to establish reliable cause and effect relationships between radiation exposure and resulting human detriment. In addition, in the event of an unanticipated radiation incident, such as a terrorist's radiation dispersal device or an accident at a nuclear facility, though a biopsy and tooth restoration technique has been developed, there is presently no means of triage dosimetry that can process mass casualties rapidly and non-invasively.

Of all living tissues, dental enamel is the only one that retains, essentially indefinitely, its radiation exposure history. The absorbed dose is stored in the form of long-lived free radicals (electrons freed by ionizing radiation, and subsequently trapped in lattice defects of the biological crystal hydroxyapatite).These free radicals have, historically, been detected using electron paramagnetic resonance (EPR). For adequate sensitivity, EPR requires a large laboratory magnetic field, and hence is an in-vitro, invasive technique requiring enamel obtained from discarded teeth. There have been, however, recent advances by Swartz in miniaturizing the EPR spectrometer and detecting free radicals in enamel of teeth non-invasively.

Since optical technology is amenable to miniaturization, a search for optically stimulated luminescence (OSL) in dental enamel was begun by this group. Godfrey-Smith and Pass (1997)[1] first reported a dosimetric effect using OSL in dental enamel. A time dependent OSL was observed under IR and green photon stimulation in gamma-irradiated samples of human dental enamel.

OSL of dental enamel can become the first non-invasive, simple, reliable, and portable means of retrospective radiation dosimetry in humans. The technique has been patented[2] and has recently received recognition, for its promise of rapid, non-invasive radiation dosimetry[3], through Department of Homeland Security funding of research by the Oakridge National Laboratory. This report will discuss potential obstacles to achieving portable, non-invasive OSL enamel dosimetry. Obstacles include low sensitivity, optical bleaching by sources of light external to the oral cavity, normalizing dose, signal fading, variations in opacity of enamel and in sensitivity to radiation-which can make it difficult to calibrate dose and establish a dose response curve.

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Design of Tooth Dosimetry Magnets

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Following an event in which a human population has been exposed to ionizing radiation, for reasons of triage it is important to determine the dose that each individual has received. This should be done in the field and rapidly. Presently there is no device that is suitable for doing this.

It is well known that, as a direct result of its interactions with molecules, ionizing radiation creates unpaired electron species and that, if they are generated in an appropriate matrix such as the hydroxyapatite component of teeth and bone, some will be stabilized for long periods of time. The effect is cumulative and it has been shown that electron paramagnetic resonance, EPR, spectroscopy techniques can be used to assess integrated radiation exposure in such a medium. Thus it may be possible to exploit such techniques to create a portable post-exposure radiation bio-dosimeter.

The magnet generating the background magnetic field, essential for enabling the EPR process, can in principle be any one of several types but the need for portability and ease of use in the field influences the selection; electrical power, size and weight should be minimized, as should the need for servicing.

Magnets of three types, resistive electro-magnets, high-temperature superconductor, HTS, electro-magnets, and permanent magnets, were designed to assess their suitability for this application. In all cases the central magnetic field was set at 0.04 T, (1.2 GHz), and the magnetic field inhomogeneity at 1000 ppm peak-to-peak over a spherical volume of diameter 3 mm to 15 mm depending on the particular design. Six designs were topical and one was intra-oral. Power consumption for the resistive magnets was 0.6 and 2.7 kW with corresponding conductor masses of 0.8 and 4.3 kg. The HTS magnet designs operated at between 64 K and 70 K with corresponding HTS masses of 2.7 and 1.8 kg. The topical permanent magnet designs had masses of 1.4 and 3.1 kg and the intra-oral permanent magnet had a mass of 7.4 g.

The study results suggest that resistive magnets are undesirable because of the high electric power demands and the need for water cooling. The HTS magnet designs suffer because of the need to maintain their low temperature environment using a cryostat and a cryocooler. Permanent magnets are the least demanding in terms of maintenance and services and may be miniaturized as exemplified by the intra-oral design. A detailed magnetic design comparison will be shown.

Overview of the *L*-band EPR dosimetry results *in vitro*. Optimization of conditions of data collection and development of software for data collection and analysis in emergency *in vivo* dosimeters.

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In vivo dosimetry has been the focus of our recent research and development activities. It is based on *L*-band electron paramagnetic resonance (EPR) spectroscopy of the radiation-induced permanent radical centers within tooth enamel of human teeth *in situ*.

An extensive database of *in vitro* results has been collected to date and is continuously growing. Spectra collected for a large number of isolated teeth at *L*-band frequencies (near 1200 MHz) show the well known presence of a background, or native EPR signal in non-irradiated teeth. UV, X-ray, beta, and gamma-ray (e.g., ^{137}Cs) irradiation produce a characteristic permanent signal distinguishable at *L*-band from the native one. These initial results have convinced us of the suitability of our approach and the feasibility of a goal of dose determination with precision of *ca*. 20 cGy. We are now beginning measurements in healthy volunteers for effective determination of the native signals and, in those subjects whose dentition is suitable, for measurements of irradiated teeth set in a partial denture.

In this initial period we became aware of the need for optimization of data collection parameters. Particularly, a range of values for the applied RF power (and, consequently, B_1 field intensity) and Zeeman field modulation amplitude have been investigated for their potential to increase the signal-to-noise ratio and to lower the limit of detection and standard deviation of the measurement. In experiments *in vitro*, the intuitively obvious setting of experimental parameters at, or near, maximum values of power and modulation amplitude have been observed to lead to prominent microphonic distortion of the baseline. Considerable effort has been dedicated to identification and amelioration of the sources of such detrimental effects.

Signal averaging has been explored extensively in collecting of our dosimetric data. Currently, satisfactory signal-to-noise ratios (SNR) can be obtained in approx. 5 minutes. Adaptive data collection routines are under development that continuously evaluate the achieved SNR, monitor scan quality and, using approaches such as median filtering, eliminate statistical outliers that would heavily bias the averaged output.

Our EPR data have been collected based on statistically-justified protocols. Data analysis algorithms have been design to allow rapid dose prediction from available information on the nature of EPR signals in teeth. Data acquisition software is being developed using the National Instruments LabVIEW platform. This allows rapid interfacing with EPR bridges and detectors. Additionally, implementation of robust analysis algorithms is facilitated.

Design of TE011 cylindrical and spherical cavities at 200 MHz for In-vivo Human Dosimeter

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A conceptual design of measuring signal of whole body at a lower frequency of 200 MHz by scaling up the size of the TE011 cavity to a few meter in the diameter is presented following a brief review of in-vivo tooth dosimeters developed in Osaka University.

The minimum detectable dose of human tooth enamel was about 1 mGy for separated tooth enamel using an X-band TE111 cylindrical cavity with a high Q = 10,000. An ESR dosimeter to determine the dose of a tooth without extraction was constructed using the permanent magnet of Nd-B-Fe alloy (Neomax) and cavities with an aperture at an X-band frequency (Ikeya and Ishii, 1989; Ishii and Ikeya, 1990). The minimum detection level was high since inhomogeneous magnetic field broadened the signal. The lower limit with a cavity with a slit of 3x10 mm was several Gy at the S/N = 1 level without signal averaging and ~ 2 Gy with 100 sweep data accumulation using a commercial ESR spectrometer (Yamanaka et al., 1993).

One can use the oscillators and detection systems of a conventional NMR or MRI machine. The external magnetic field as well as the field gradient and modulation to the head may be applied by the current in the straight rods or pipes in the cavity room similarly as was used to apply the intense field gradient for CT-ESR microscope at an X-band frequency (Furusawa and Ikeya, 1991). Thus, large size in-vivo cylindrical TE011 cavity using the double frequency modulation with lock-in-amplifiers will allow to detect signals in teeth in human dosimetry and radicals in human tissue in clinical uses. The disadvantage of observing signals of Fe3+ and the hyperfine lines Mn2+ as a single broad band cannot be avoided at a low frequency where the Zeeman energy is smaller than the hyperfine energy as shown in the spectra of Mn2+ in MgO atdifferent frequencies. The sensitivity of lower frequency apparatus may be improved by a noble noise rejecter.

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ESR Analyses for the Paleolithic Hominid Site at Obi-Rakhmat, Uzbekistan: Solving a Dating Controversy

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In the Tien Shan Mt., northeastern Uzbekistan, the Obi-Rakhmat rockshelter has yielded newly discovered hominid teeth and cranial fragments from at least two youths showing both modern and archaic traits. Metric comparisons for the hominids from Obi-Rakhmat suggest a resemblance to those from Pe tera cu Oase, Romania, identified as anatomically modern *Homo sapiens*. These hominids occurred associated with an abundant, blade-based industry having both Middle and Upper Paleolithic characteristics and a rich faunal assemblage in typical cave fill sediment. The archaeology at Obi-Rakhmat shows many similarities to the initial Upper Paleolithic complexes in Eurasia, such as at Bohunice, Boker Tachtit, and Kara-Bom. The fauna and pollen indicate a steppe environment similar to that in the region today.

Because ESR can absolutely date enamel from 5 ka to 5 Ma, eight bovid teeth were dated by standard and isochron ESR to resolve the 50 ky discrepancy between the ^{14}C and $^{234}Th/^{234}U$ ages for Obi-Rakhmat. All the dentine contained high U concentrations, up to 150 ppm. External dose rates of 800-1500 μGy/y were calculated from volumetric sediment geochemistry for all layers and mineralogies within 30 cm of the teeth. Teeth from Layer 13 averaged 64 ± 1 ka assuming linear U uptake, while that from Layer 14.3 averaged 68 ± 2 ka, and those from Layer 21.2 averaged 87 ± 3 ka. Isochron analyses suggest that the teeth did experience linear uptake with < 5% secondary U remobilization. These results indicate that deposition of the archaeological deposits occurred when stalagmitic deposition had slowed in the cave, during Oxygen Isotope Stages (OIS) 5a-4, as climates were generally cooling, but beginning to fluctuate more wildly. Assuming a constant sedimentation rate of 22 cm/ky, the hominid remains date to ~ 72 ka, and the MP-UP transition to ~ 66-58 ka.

Chemical Process to Separate Magnetite Particles in Pottery Samples for ESR Dating

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Most ancient pottery is made of local clay material, which contains relatively high concentration of iron. The powdered samples are usually quite black, due to magnetite, and, although they can be used for TL dating, the TL signal is not as good as for lighter-colored materials. For ESR measurements, the huge signal due to spin-spin interaction hides any other signal. As consequence, ESR dating can not be used, since iron signal do not depend on radiation dose. In such a case, the density separation method using hydrated solution of sodium polytungstate [$Na_6(H_2W_{12}O_{40}).H_2O$] becomes useful. The density of magnetite is 5.16g/cm^3, of the sodium polytungstate, 3,23g/cm^3 and of quartz 2,65g/cm^3. However, the sodium polytungstate is very expensive in Brazil. Thus an alternative method for eliminating this interference is proposed..

We developed the following chemical process to eliminate ca. 90% of magnetite. A sample of powdered ancient pottery was treated in a mixture (3:1:1) of HCl, HNO_3 and H_2O_2 for four hours. After that, it is washed several times in distilled water to remove all acid matrix. The original black sample becomes quite white. The resulting material was analysed in ICP-MS system, showing that the iron content is reduced by a factor of about 9. In ESR measurements it was observed that, while a non-treated natural ceramic sample shows a broad spin-spin interaction signal, the chemically treated sample presents a signal (narrow) in g = 2.00 region, possibly due to a radical of $(SiO_4)^{4-}$. This signal increases in intensity under γ-irradiation. ESR and TL dating of pottery are under way and will be presented later on.

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ESR Analyses for the Paleolithic Site, at Attirampakkam, India: Clues to Complex U Uptake and Paleoenvironmental Change

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At Attirampakkam, in Tamil Nadu, India, a stratified open-air Paleolithic site yielded Acheulian artefacts from Pleistocene fluvial sediment. Near the Kortayallar River and the Indian Ocean, Attirampakkam is prone to sealevel fluctuations, channel avulsions, and lateral river channel migration. Interbedded in laminated clay and ferruginous gravel beds, Attirampakkam is the type site for the Mandras Handaxe Tradition of the Indian Acheulean (Lower Paleolithic). The site has never been successfully dated. The archaeological layers have yielded six Pleistocene vertebrate fossils, including the three teeth dated here.

Electron spin resonance (ESR) can date tooth enamel between 10 ka and 5 Ma in age. Few ESR studies have used teeth from open-air sites, which often suffer from severe, pervasive, and rapid diagenetic alteration and weathering, producing teeth that are difficult to prepare for ESR analysis.

All the available teeth were analyzed ESR, and two were prepared for ESR isochron analysis. Despite their diagenetic alteration, the teeth yielded 20 independent ESR enamel ages, and three ESR isochron analyses. Diagenetic alteration features in two teeth indicated rapid submergence in quiet saline to hypersaline water, following a short subaerial exposure, while the third remained constantly buried under reducing conditions. Hence, their dates also indicate the timing for the geochemical and sedimentological events affecting the site. All the geochemical, standard and isochron ESR analyses indicate that all the teeth experienced at least three independent U uptake events during diagenesis, including two that occurred after burial. Assuming linear U uptake (LU) adjusted for the multiple U uptake events, the teeth averaged 45-50 ka. Numerous animal burrows, the geochemistry of the teeth's coatings, and artefact displacement, suggest that the teeth were reworked from the Middle or Upper Palaeolithic layers higher in the site which, therefore, correlate with Oxygen Isotope Stage 3.

Decay of the ESR signals in quartz by the high speed friction experiments: Basis for dating of fault movements

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The ages of fault activity have been obtained by applying the ESR dating method using quartz in fault gauge. However, the mechanism of zeroing of the signals by faulting is not well understood yet. In the present study, high-speed friction experiments with the conditions closer to actual fault movements were conducted. The decrease of the signal intensities by this experiment was compared with those of heating experiments, so that the temperatures during high speed friction experiments were estimated.

The high speed friction experiments were performed at Kyoto University using quartz grains extracted from Mannari granite, Okayama, Japan. The grains were irradiated by gamma rays to 1.5kGy prior to the experiment. Quartz grains were placed between two gabbro columns of 25mm diameter with a tephlon sleeve. One column rotate with speed of 300, 600, and 900 rotations per minute until it reaches 300 rotations when vertical load of 30kgw was applied. The sample was powdered. Each sample was separated into three parts, center, middle, and out, according to the position between the columns. The signal intensities of ESR signals were measured by ESR. The isothermal heating experiments were done at six different temperature with ten steps of heating duration.

On the Influence of Carbonate Calcite Contamination on ESR Dating

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Calcite is a material widely used for EPR dating. The ages of stalagmite and other deposits have been found by measuring the concentration of the radical CO_2^- as function of the artificial dose. Calcite formations are usually developed by dissolution of limestone ($CaCO_3$) by water containing carbon dioxide (CO_2), followed by reprecipitation as the water evaporates. Stable free radicals are generated in calcite by interaction with ionizing radiation. Usually there are at least two radical species present in the spectrum, one axial and another isotropic. The EPR signal has a relatively small growth rate with time, since the radioisotope content of the material and its surroundings is generally low. The low growth rate implies lower precision in calcite than, for example, in tooth enamel. One should also consider the possibility of sample contamination by solid carbonate particles which would introduce older carbonate ions into the crystallizing deposit. This model describes the influence on the archeological dose (AD) of the presence of 'old' carbonate in a sample.

Interlaboratory comparison on Tooth Enamel Dosimetry on Semipalatinsk Region: Part 1, General View

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Since ESR/EPR method was found to be useful for retrospective dosimetry of human teeth, it has been the issue how accurately the doses can be obtained. There have already been three interlaboratory comparison projects organized where successful results were obtained. However, which factor gives variation in the nominal doses is still unknown. In the present intercomparison, the human teeth from actual radiation accidents were analyzed in different laboratories to see how close the obtained doses are to each other. Each laboratory used the same measurement condition together with optionally their own measurement condition. This will tell the difference in using different spectrometers, measurement conditions, and data processing. Irradiated tooth samples will also be examined to check if the methods work properly.

The 14 tooth enamel samples were provided from Semipalatinsk region received some amount of accidental doses estimated to be from 50 to 500 mGy. For the calibration for these samples, a set of eight irradiated samples are prepared from pooled enamel obtained from teeth collected in control territory of Semipalatinsk region. In addition, ten test samples from five Japanese molar teeth are provided. Each tooth was cut in half and five halves were irradiated to known doses between 100 and 300 mGy and the other halves were not as it was done in the 3rd intercomparison. The results of the measurements will be presented.

Interlaboratory comparison on Tooth Enamel Dosimetry on Semipalatinsk Region: Part 2, Effect of Spectra Processing Procedure

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In order to reveal effect of the EPR spectra processing procedure applied to spectra measured in different conditions, spectra of the same enamel samples measured in different laboratories were distributed among other participants of the intercomparison. Spectra measured in different laboratories are processed for dose determination by some of participants using their procedures. Among the samples there were samples irradiated in known doses (calibration samples) and samples irradiated in doses unknown by participants (testing samples). As criterion of quality of the spectra processing, mean square deviation of radiation-induced signal intensity from the regression line of dose dependence is used for the calibration samples and mean square deviation between experimental and nominal doses for the testing samples. Analysis of the results of dose determination obtained with the use of the same spectra processing procedures applied to spectra measured in different laboratories will be presented.

The Assimilation of Cytogenetic, ESR and Biochemical Assays For Highly Exposed Victims

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An inter-comparison of data from highly irradiated accident victims was carried out in order to assess the level of agreement between cytogenetic, ESR and biochemical assays where two or more parameters were investigated from the same subject. The study group comprised 42 subjects who exhibited acute radiation syndrome (ARS) in a consequence of radiation accidents during the period 1961-2001. They were overexposed as a result of the Chernobyl accident (10 subjects), nuclear submarine accidents in the Russian Navy (24 subjects) and various accidents with industrial/research radiation sources (8 subjects).

The following assays were used: conventional dicentric and FISH translocations in blood lymphocytes, ESR measurements on tooth enamel and molecular-biochemical analysis of the oxidative status and DNA structure in leucocytes and mononuclear cells. The initial dicentric and later translocation frequencies were measured for all study subjects. The ESR and biochemical assays were applied only long after irradiation with 24 years delay at the average. In total, during medical treatment 10 teeth were collected for ESR analysis and 17 subjects were examined by biomolecular assays. The latter were colorimetric (with a methyltetrazolium compound, MTT-test) and fluorimetric assays for the measurement (with dichlorodihydrofluorescein diacetate) of reactive oxygen species (ROS) in blood cells. It was assumed that the level of ROS production inferring a DNA structural changes in cells can reflect the degree of late effects induced by ionizing radiation.

Individual doses based on yields of late translocations were in good agreement with initially estimated doses based on dicentrics and with those obtained by ESR spectrometry of tooth enamel. Moreover a reasonable correlation was found between the frequency of late translocations and the severity grading of the ARS exhibited immediately after whole-body acute doses assessed as being within the range 1-10 Gy. Biochemical indices of later blood samples did not coincide with the grade of ARS, but some correlations were noted between biomolecular and cytogenetic data. The significance of radiation-induced genomic instability on these assays for irradiated victims is discussed.

Dosimetric properties of the incisor teeth. Part 1: Influence of sample mass on dose estimate

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The method of EPR tooth dosimetry has reached a certain level of perfomance. Multi-center intercomparisons have validated the system and international recommendations have put the basis for standardization of the method. However, both intercomaprison and recommendations have been limited to molar teeth. The question arises whether the dosimetric properties of incisors are similar to those of molars. Absence of knowledge of the EPR dosimetric properties of incisors is critical in epidemiological studies because this leads to their exclution, which can form a large part of the teeth contained in sample banks. Two issues can produce differences in the dosimetric properties of deciduous and molar teeth. The first is that the available sample mass is much smaller for incisors than for molars. This is not only due to the incisor volume which is intrinsecally smaller than molars, but also to the common recommended practice of rejecting the front half of incisors because of possible presence of free radicals induced by solar UV. The other issue is that the cristallinity of the incisors has been hypothesised different from that of molars, although a definitve proof has not been provided. This difference in crystal structure could reflect in different spectrum shape and radiation sensitivity.

The present paper is the part 1 of a series of investigations focused to the analysis of the dosimetric properties of incisors. This part is aimed to evaluate the influence of mass on the quality of the EPR spectrum. The part 2 will be dedicated to the comparison of the radiation sensitivity between enamel from different parts of incisor and molar teeth.

This study was performed on teeth of donors belonging to two uncontaminated populations and the EPR measurements were performed with two different types of microwave cavitiy and EPR instrumentation. The sample mass varied between a few and 100 mg. The estimated dose showed a decreasing trend with mass. This effect was found to be related to EPR signals from impurities present in the resonant cavity and sample tube and overlapping to the dosimetric signal. It will be shown that correction by an appropriate function accounting for the cavity signal intensity with mass increases the accuracy of dose estimate of small teeth.

A method to differentiate between the levels of ESR signals induced by sunlight and by ionizing radiation in teeth from atomic bomb survivors

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Electron spin resonance (ESR, or electron paramagnetic resonance, EPR) analysis of tooth enamel is an effective method for the retrospective estimation of individual radiation doses. One problem with this technique is that the observed ESR signal may include a contribution from ultraviolet light (UV) exposure from sunlight, especially in front teeth. Thus, it has been desirable to find ways to estimate the UV effect in the total signal so that the net ESR dose from ionizing radiation can be determined.

To examine this issue, we measured 96 teeth of various types, but with buccal and lingual parts separately, from control subjects of atomic-bomb survivors.

We found that, except for molars, the mean ESR-estimated dose of the buccal halves was, on average, nearly twice that from the lingual side, which indicates that the UV-induced lingual dose equals to the difference between the two halves.

Using these corrections for UV exposure to front teeth that had been exposed to both ionizing radiation and UV, it was found that the estimated radiation doses closely approached the previously estimated ESR dose to molars from the same donors, or to the estimated dose arrived at with cytogenetic methods.

We concluded that, in using ESR to estimate radiation dose, measuring molars is the first choice, but if only front teeth are available, separate measurements to the buccal and lingual parts can provide an estimation of the mean UV contribution to the ESR-determined dose.

Lower Bound Of Enamel Radiation Sensitivity To Neutrons

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Radiation sensitivity of tooth enamel to neutrons *in vitro* is very weak, at least for neutron's energy up to several MeV. In practice tooth enamel is exposed to ionizing radiation as a part of human body. In this case radiation yield of paramagnetic centers in tooth enamel (and radiation sensitivity as well) should be determined by secondary photons produced mainly in reaction of radiation capture - ${}_1H^1(n, \gamma){}_1H^2$ (E = 2.23 MeV) - which is the most important one contributing significantly to dose in the soft tissue, and than only in the thermal and near-thermal energy region. In this work the role of secondary photons to dose formation at neutron irradiation is investigated.

Mathematical human heterogeneous phantom was involved into Monte Carlo simulation with newly defined dental region. Calculation of photon and total absorbed dose for whole body, different organs/tissues, tooth enamel and dentine was done for monoenergy neutrons of different energy incident on human phantom in ISO geometry. These data can be considered as a lower bound of enamel radiation sensitivity to neutrons (Fig. 1). Total absorbed doses for different organs/tissues were found to be within 10 % deviation from presented in ICRP Publication 74 (1996). Calculated data are also supported by coupled experimental/calculated investigation of enamel irradiation by reactor neutrons in tissue-equivalent phantoms.

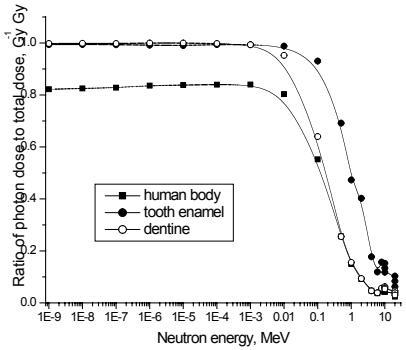

Fig. 1. Ratio of photon absorbed dose to total absorbed dose at neutron irradiation of human heterogeneous phantom in ISO geometry.

Enhancement of the EPR sensitivity of tooth enamel to neutrons at irradiation in the human head phantom

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Dose response of human tooth enamel to fast neutrons at irradiation in the air is known to be very small because of low content of protons in enamel via which transferring energy from neutrons takes place. However, at irradiation of enamel surrounded by soft tissues secondary ionizing particles arising such as photons at nuclear reaction $H(n,\gamma)H^2$, E_γ=2.23 MeV and high energy scattered protons. These particles increase dose absorbed in enamel and enhance ionization leading to increasing of the EPR signal. This work is aimed on investigation of effects of proton containing surrounding materials on the EPR response of enamel to neutrons.

Irradiation was performed by neutrons produced by neutron generators with average energy of 1 MeV (in RIRBM) and 14 MeV and (in MRRC). A pair of dosimeters – tissue equivalent and low neutron sensitive ones, was used for determination of total tissue equivalent dose, neutron and gamma components. Method of EPR spectroscopy was used for determination of absorbed dose in enamel according to calibration using a ^{60}Co source and tissue equivalent dosimetry.

At irradiation by 1 MeV neutrons in a 30-cm cubic phantom filled by water at depth of 2, 9.5 and 15 cm it was found that dose in enamel correspond respectively to (12±5)%, (80±10)% and (110±20)% to total tissue dose. The observed enhancement of dose in enamel is caused by secondary photons. At irradiation without phantom doses in enamel are less than 2% of tissue doses. Effect of scattered protons to dose in enamel for 1 MeV neutrons is found to be within experimental uncertainty (less than 5% of total tissue dose).

For 14 MeV neutrons, at irradiation in the air dose response of enamel is corresponding to (13±4)% of total tissue dose. Effect of scattered protons is investigated at irradiation of different grain size enamel in the air and in water filled tubes. Dose in enamel was found to be (15±6), (19±8) and (21±7)% of total tissue dose for average grain sizes of 1.4, 1.0 and 0.7 mm respectively. At irradiation in the 30-cm cubic water filled phantom at the distance of 2 cm from the source dose in enamel is 24±8% of total tissue dose. Effect of dose enhancement in enamel due to scattered protons is higher in comparison with 1 MeV neutrons. Depending on the enamel grain size, it varies from 2% of total tissue dose for 1.4-mm grains to 8% for 0.7-mm grains.

Neutron and photon doses in dosimeters were calculated using MCNP-4B Monte-Carlo code taking into account neutron, photon, electron transport and secondary photons. Geometry of the source and an energy angular distribution function were taken into account at calculations. Calculated photon and neutron components to doses are agreed with experimental ones, which validate the calculations. Basing on that, calculations of doses in enamel are performed for another geometry of irradiation and another neutron energies relevant for practical application. Effect of scattered protons was taken into account as correction coefficients basing on the experimental data.

Temperature-stimulated transformation of radiation induced CO_2^- in tooth enamel plates

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The EPR spectrum near g=2 in irradiated tooth enamel is widely used in retrospective EPR dosimetry. It is known that this spectrum is caused mainly by two types of CO_2^- radicals. One type of the center is the bulk oriented CO_2^- radical which occupies B position in the hydroxyapatite crystallites lattice. The second type centers are the disordered species which are supposed to localize on the surface of apatite crystallites and/or in organic matter.

We report the study of the contributions of the oriented and disordered CO_2^- to γ-radiation induced EPR spectrum of tooth enamel plates annealed at different temperatures. The samples of clinically sound enamel were cleaned with the help of dental instruments and nonabrasive polishing paste. Small enamel plates of approximately 1×2×3 mm were cut from the surface of tooth and irradiated by ^{60}Co γ-rays at room temperature. The absorption dose was estimated to be 4 kGy. Isochronal annealing was performed in a muffle furnace in air at different temperatures up to 360^0C.

EPR measurements were carried out using X-band spectrometer at room temperature. The total amount of paramagnetic centers was determined relative to a $MgO:Cr^{3+}$ standard sample with a known number of spins.

It is found that thermal annealing causes the decreasing of total quantity of paramagnetic defects while the amount of oriented CO_2^- radicals is increased. This phenomenon was explained by the transformation of disordered CO_2^- into oriented ones. Such transformation of radicals could take place if both radicals are located in the same structural position, namely, B site in hydroxyapatite lattice.

The experimental results are explained assuming the existence of two different precursors. These precursors are CO_3^{-2} molecular ions located in the B position of apatite lattice. The difference between the precursors is either presence or absence of a crystal lattice defect nearby. Correspondingly, the irradiation of the enamel results in the creation of two CO_2^- types: the oriented centers in defectless lattice surrounding and the disordered centers with a lattice defect nearby. Thermal annealing leads to the reduction of the amount of lattice defects, and thus to the transformation of the disordered radicals to the oriented ones.

Analysis of EPR tooth enamel spectra exposed to combined radiation and mechanical effects

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It is known that long-living free radicals are formed in tooth enamel with separate radiation and mechanical effect. The studies of their combined effect on tooth enamel have not been conducted so far. The spectral analysis showed that mechanical and radiation signals were found in one sufficiently narrow field of EPR spectrum, and g-factors of their maxim coincided. Experiments of the combined radiation and mechanical effect on tooth enamel have been performed with two possible modelled situations: the radiation and then the mechanical effect and vice versa. In the first series of experiments, the whole tooth was irradiated with the dose of 30 Gy. Then enamel was separated from dentine in one half of the tooth using borers with low speed of rotation (<30.000 rev/min) with water-cooling. The second half of the tooth was a preparated using borer with high speed of rotation (≥300.000 rev/min) with water-cooling, i.e. the conditions of mechanical effect in the process of dental treatment were reproduced. The obtained results showed that in EPR enamel spectra exposed to radiation and mechanical effect (high-speed effect), increased dosimetric signal was observed as compared with EPR spectra only exposed to radiation (low-speed effect). This testifies to the fact that in the process of mechanical effect, annealing of radiation-induced radicals due to local overheating of enamel during the process of friction does not occur. In the second series of experiments, the teeth under study were divided into halves. After that, one half of the tooth was preparated at low, and the second half – at high speed of rotation. Tooth enamel preparated with high-speed borers lead to the formation of mechano-induced signals. Irradiation of enamel samples with the exposure dose of 30 Gy caused the formation of a typical radiation signal in EPR spectra in both cases. The intensity of such a signal in spectra of samples preliminarily subjected to high-speed mechanical effect exceeded the signals intensity of samples preparated at low speed of rotation. The observed increase in the dosimetric signal in tooth enamel spectra exposed to a combined effect in both series of experiments occurs as a result of superposition of the radiation- and mechano-induced signals. Thus, for adequate assessment of individual absorbed doses by EPR dosimetry tooth enamel, it is necessary to take into account the contribution of mechano-induced paramagnetic centers formed in the process of dental treatment.

Radiation Dose Estimates and Risk Assessment in Atomic-bomb Survivor

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Studies of atomic-bomb survivors and their children in Hiroshima and Nagasaki carried out by the Radiation Effects Research Foundation (RERF), and its predecessor, Atomic Bomb Casualty Commission (ABCC), are the centerpiece in radiation risk assessment. The large number of cohort subject drawn from people in the two cities, both men and women, exposed to radiation at different times in life, and the comprehensive nature of long-term follow-up are essential for understanding the temporal patterns of radiation-related cancer and other disease risks. Equally important is the availability of reliable and well-characterized individual dose estimates, which are the requisite for quantitative assessment of the risk. The history of ABCC/RERF research indicates a continuing process reflecting evolution in goals and interests of health effect research intertwined with that of the survivor dosimetry. While the earliest ABCC research interests were descriptive, focusing on clinical detection of diseases following exposure to the atomic bombs, the pivotal event was the launching of the unified study program in the 1950s following the Francis Committee recommendations. Under this program, epidemiology and all other research were to be conducted in the framework of the fixed cohort of atomic bomb survivors and controls, i.e., Life Span Study (LSS) cohort with the aim of investigating long-term health effects from radiation exposure. This was accompanied by the first systematic effort to develop individual dose estimates (T65D) in the cohort setting, following earlier tentative dose estimates. This included large-scale field investigations to obtain individual shielding information and other fundamental works. However, a question raised by Rossi in 1976 regarding the relative biological effectiveness of neutrons prompted the reassessment of T65D and eventually led to the development of DS86. Fundamentally different from the previous empirical approach, DS86 used the basic principles of physical interactions of individual particles and quanta of radiation and uses new data and ideas to assess shielding effects, providing organ dose estimates for 15 organs. DS86 was in use for some 15 years, during which biodosimetry markers, including FISH chromosomal aberrations, were investigated, providing useful results. One important development for risk estimates has been a series of studies by statisticians to estimate uncertainties in survivor dose estimates, and this eventually led to a practical system to adjust for random errors in the dose estimates, which is now routinely used routinely for risk analyses. New speculation and controversy raised about neutrons motivated the examination of DS86, although, contrary to expectation, DS02 did not produce large changes from DS86 in neutron or gamma doses at distances relevant to survivors. The approach taken for DS02 was comprehensive and addressed a number of issues that had been identified during the 15-year experience with DS86. In view of the long-lasting effect of radiation exposure on the risk of cancer and other diseases, as demonstrated by analyses of the LSS data, the survivor studies will continue. While major revisions in dose estimates are unlikely in future, there is no doubt that a continued effort will improve and refine the dose estimates, as follow-up of the LSS in the next few decades will provide critical information on the temporal pattern of the radiation risk.

A Focus on Cytogenetic Dosimetry

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This paper will provide an introductory overview to biological dosimetry by cytogenetics.

The long established dicentric assay in lymphocytes is still the most frequently employed method. A recent development has been its international standardisation and this is to be followed up by a standard for its rapid deployment in triage mode. Several groups have organised mutual assistance networking as a means of effective surge response to a major accident or radiological terrorism. The micronucleus assay is another option for dealing with large numbers of samples; each assays has its pros and cons.

One long recognised drawback with dicentrics is the evaluation of very high doses. This arises from the tendency of the dose response curve to saturate and also the impaired ability of cells to reach metaphase in culture. A significant development with this problem has been the use of chemically induced premature chromosome condensation combined with the scoring of rings.

Retrospective dosimetry, to be employed over the time scale when the dicentric signal has reduced, is now possible by the FISH method which allows detection of 'painted' translocations. A consensus has been reached among a number of European laboratories on how best to use this assay. The important features concern a) what chromosomes to paint, given that for routine application it is sufficient to paint only a part of the genome; b) what to score, i.e., which metaphases to select and which aberration types best relate to retrospective dose; c) how well do so-called stable translocations really persist on a time scale of many years; d) the variability of the control frequency of translocations and its dependency on donor age; e) considerations of in vitro calibration, and finally, f) how sensitive is the method in detecting previous exposures, bearing in mind that its most frequent application will be for acute low doses that passed unnoticed, because they caused no obvious clinical signs, or high doses received at low dose rates.

Needs for a standardisation of biological dosimetry by cytogenetics in expertise situations and population triage

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The wide use of radioactive sources and X-rays, for medical, industrial, agricultural, research and military purpose increases the risk of overexposure of occupationally exposed persons and individuals of the general population. Biological dosimetry, based on the study of chromosomal aberrations, mainly the dicentric assay, has become a routine component of the accidental dose assessment. Experience of its application in hundreds of cases of suspected or proven overexposures has proved the value of the method and also defined its limitations.

The technique is incorporated into radiation protection programmes of several countries and it has even acquired a medical-legal status, to confirm or discount a suspected radiation exposure. Radiation accidents are fortunately few so that the number of laboratories performing biological dosimetry in an official capacity is, generally, only one or two per country. By contrast, the absence of real concurrence underlines the needs for such an important and widely applied technique has to rest on solid bases, to assure its credibility. Therefore, it was decided to provide a guideline to all laboratories in order to perform dicentrics assay in the most standardised conditions. While recommendations documents are provided by different organisations such as IAEA, an ISO standard was judged most appropriate to address the critical aspects of the use of the dicentric assay as a biodosimeter. This 19238 ISO standard published in 2004 provides criteria for quality assurance and quality control, evaluation of performance and the accreditation of biological dosimetry by cytogenetics service laboratories. It introduces the formal comparison of the results obtained in one laboratory to another one, particularly in case of an international collaboration or intercomparison. Finally, each new laboratory must get from this standard the most useful information to perform dicentrics assay in the best experimental and reproducible conditions.

In addition, a potential for nuclear and radiological emergencies involving mass casualties from accidental or malicious acts is emerging from the worldwide situation. Cytogenetic triage, i.e. the use of chromosome damage to evaluate approximately and rapidly radiation doses received by individuals, could be appropriate in order to supplement the early clinical categorization of casualties. The dicentric as well as several alternatives cytogenetic – chromosome aberration based assays (i.e., premature chromosome condensation, micronuclei, etc.) are useful for this purpose. However, this event can also exceed the resources from the locally involved biological dosimetry laboratory, requiring the intervention of other laboratories within the constitution of a network. Several biodosimetry laboratories have independently and successfully performed rapid dose assessment in mass casualties'incidents or exercices. Their approaches included using pre-planning, reagent stockpiling, simplified sample processing, automation, modifying some of the ISO 19238 scoring criteria, and networking with other expert laboratories. Building upon their experience, a new ISO international standard is in progress to define criteria for performing quality assured cytogenetic triage.

§ - *And the other members of the ISO Working Group 18 on biological dosimetry.*

International Study of Translocations in Control Populations

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Biological monitoring of radiation dose can contribute important, independent estimates of exposure for individuals and populations, especially when physical measurements of radiation exposure are unavailable. Translocations have been the most widely applied biological marker of past radiation exposure in epidemiologic studies, because of well-characterized radiation dose-response curves and the persistence of translocations that can be detected many years later. Establishing baseline levels of translocations will contribute to the usefulness of this technique in cases of accidental radiation exposure.

It is well accepted that the frequency of chromosome aberrations increases with radiation exposure and age, but the effect of gender, ethnicity and lifestyle factors (such as cigarette smoking) on background translocation yields is not known with certainty. Pooled analyses of translocation data in unexposed individuals have been conducted, but the largest study to date included only 385 persons. Background aberration frequencies were overwhelmingly influenced by age, but the effect of age as modified by gender and cigarette smoking remains unclear. We sought to expand the number of contributing laboratories with the goal of establishing control levels of translocation frequencies by age, gender, ethnicity and smoking status.

Fifteen laboratories in North America, Europe and Asia contributed translocation frequency data on 1,957 unexposed individuals, with a minimum of 200 cell equivalents (CE) per individual. Ages ranged from newborn (cord blood) to 85 years. The study population was 37% female, 40% reported ever smoking, and 77% were Caucasian, 13% Black, 8% Asian, and 2% were other ethnicity. Age was the strongest predictor of translocation frequency ($p<0.001$). The mean number of translocations was 0.03/100 CE (95% confidence interval=0.02-0.04) for newborns and 1.7/100 CE (95%CI 1.4-2.0) for subjects 75 years and older. An analysis of the effects of gender showed that translocation frequencies per 100 CEs were similar for men and women up to about age 50, when they diverged, with women having higher frequencies than men. Smokers had higher translocation frequencies than non-smokers ($p < 0.001$) after adjustment for gender, ethnicity, and laboratory. We noted significant variation by laboratory in all analyses. We were unable to separate the effect of ethnicity on translocations from inter-laboratory variation. More work is needed to understand the different types of age responses in different populations.

FISH Chromosome Analysis of Sellafield Radiation Workers with Internal Deposits of Plutonium

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Studies of the health effects of occupational exposure to radiation require reliable dosimetry data for the findings to be correctly assessed and it is essential that radiation doses determined by physical dosimetry can be verified and validated. FISH chromosome analysis for translocations is a well established retrospective biodosimetry technique for low LET exposure but the interpretation of chromosome aberration yields following high LET exposure has been more difficult. *In vitro* studies have suggested that the most likely consequence of a direct traversal of a nucleus by an alpha particle is the formation of a complex chromosome rearrangement and it has been suggested that these could be a marker for high LET exposure. However, the majority are nontransmissible and therefore following alpha particle irradiation of the bone marrow by deposited plutonium very few chromosomally damaged cells are expected to survive and little chromosome damage should be seen in descendant peripheral blood lymphocytes.

Chromosome analysis using a single-colour FISH technique was undertaken on a group of 46 retired plutonium workers with assessed bone marrow doses >60mSv, 34 of whom were categorised as having robust dosimetry and 12 for whom internal doses were considered less reliable [1]. The simple translocation frequency of $17.65 \pm 1.96 \times 10^{-3}$ per genome equivalent for the 34 plutonium workers with robust dosimetry was significantly increased in comparison with that of $10.06 \pm 1.16 \times 10^{-3}$ per genome equivalent for the unirradiated control group ($P = <0.001$) and that of $13.55 \pm 1.43 \times 10^{-3}$ per genome equivalent for the group with similar external gamma exposure ($P = 0.012$). No increases in dicentrics or complex aberrations associated with plutonium exposure were observed and it can therefore be assumed that there is little, if any, ongoing irradiation of mature lymphocytes. The translocation frequency of $12.08 \pm 1.92 \times 10^{-3}$ per genome equivalent for the group of 12 plutonium workers with less reliable internal dosimetry could adequately be accounted for by age and external dose and suggests that the internal bone marrow doses are likely to have been over-estimated.

Thus whilst no specific fingerprint for high LET irradiation could be discerned using a single colour FISH technique it is clear that *in vivo* a significant proportion of irradiated cells survive with simple exchanges, i.e. translocations, which can be passed on to descendant cells. Cytogenetic analysis can therefore make a valuable contribution to the validation of internal doses from plutonium deposition.

[1] E. J. Tawn, C. A. Whitehouse and A. E. Riddell, FISH Chromosome Analysis of Plutonium Workers from the Sellafield Nuclear Facility, Radiat. Res. (in press).

An Automated Method to Quantify Radiation Damage in Human Blood Cells

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Cytogenetic analysis of human lymphocytes is an established method for assessing the absorbed dose in individuals who have been exposed to ionizing radiation. Because mature lymphocytes circulate throughout the entire body, the dose to lymphocytes is considered an accurate surrogate for whole body exposure. Damage caused by radiation can be quantified at the metaphase or interphase stage of the cell cycle. Both methods have been used to evaluate the effects of various types of radiation on lymphocytes and both have been used to generate calibration curves. Cytogenetic methods indicate that damage to human chromosomal DNA can be detected at absorbed doses as low as 5 cGy.

The latest cytogenetic methods involve chromosome-specific DNA probes combined with fluorescence staining which allow selective "painting" of any or all chromosomes in the human complement. Although the widely used fluorescence in situ hybridization (FISH) method has replaced the time consuming analysis of banded chromosomes, it remains in a validation stage. FISH – based analysis has several drawbacks including use of a toxic chemical to denature DNA, expensive DNA probes, need for fluorescence microscopy, inability to cover all chromosomes without incurring even higher cost and recent evidence showing that chromosome translocations may be less stable than expected.

The goal of this study is to evaluate an inexpensive and simple chromosome staining method combined with digital imaging and automated karyotyping as a standardized method to detect and quantify structural chromosome aberration rates. The method covers the entire genome by screening all 23 chromosome pairs which are automatically assembled into a standard karyotype which can be quickly examined for aberrant chromosomes. A critical evaluation of this method will be performed by analyzing lymphocytes from former plutonium workers with known external, internal and bone marrow doses. We have previously examined cell samples from these workers using a FISH – based method which showed dose-dependent increases in translocations involving chromosomes 1, 4 and 12. The study will allow for direct comparison of the two methods since they will be based on duplicate samples from the same culture for each individual in the study. Preliminary results show this method may augment the dicentric assay by including a wider spectrum of cytogenetic effects.

Laboratory automation and information management for cytogenetic biodosimetry

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Cytogenetic methods can be used to assess radiation dose after accidental overexposures. Mass casualty high-dose exposures will require a large number of samples be processed for clinical triage. We have established the lymphocyte metaphase-spread dicentric assay in accordance with international protocols. However, chromosome-aberration analysis is time consuming and laborious. We will describe our efforts to increase sample throughput via automation, technology integration, and the implementation of a laboratory information management system for resources and data. The components of an automated cytogenetic biodosimetry laboratory include sample and reagent bar-code tracking, a robotic liquid handler, a metaphase harvester, a spreader for metaphase-spread preparation, an integrated slide stainer and coverslipper, a high-throughput metaphase finder, and multiple satellite chromosome-aberration analysis systems. Our studies will improve diagnostic biodosimetry response, aid confirmation of clinical triage and medical management of radiation exposed individuals.

Depleted Uranium Mutagenicity and Deletion Pattern Analysis at the Hypoxanthine Phosphoribosyl Transferase (HPRT) locus: Method to Discriminate Depleted Uranium Exposure from Other Genotoxic Poisons

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Depleted Uranium (DU) is a heavy metal used in military munitions. Limited data exist to permit an accurate assessment of risks for carcinogenesis and mutagenesis from DU embedded fragments or inhaled particulates. DU is unique in comparison to other heavy metals in that it is radioactive and emits alpha particle radiation. Ongoing studies are designed to provide information about the carcinogenic potential of DU using *in vitro* and *in vivo* assessments of morphological transformation, cytogenetic, mutagenic, and oncogenic effects. As a comparison, alpha particle radiation, gamma radiation, and other heavy metals, i.e., tungsten alloys and nickel are being examined.

To investigate the mutagenic mechanisms of DU, the hypoxanthine guanine phosphoribosyl transferase (HPRT) mutation assay was used. The mutation frequencies and mutation spectra that are induced by DU were investigated at the HPRT locus in V79 Chinese hamster cells. The mutation frequency induced by DU was approximately 22-fold higher than that observed in control populations. Both alpha particle and gamma radiation induced a 27- and 8-fold increase in mutation frequency, respectively. In comparison to control cells, tungsten alloy and nickel induced a 16- and 4 fold increase in mutation frequency. The data indicate that both the heavy metals DU and tungsten are mutagenic.

Deletion-pattern analysis of the DU-, alpha particle, gamma radiation, tungsten, and nickel mutations at the HPRT locus was done. At approximately 40%-survival level, DNA deletions were analyzed by multiplex-PCR analysis of all nine exons of 21-30 mutants. The resulting mutant deletion-pattern distributions were corrected for background mutations. Alpha particles and DU induced a larger fraction of total deletions than gamma radiation, tungsten, and Ni. The percentage of none, partial, and total deletions for alpha particles and DU was similar with a slightly higher amount observed in alpha particle mutants. In contrast, the fraction of deletions was similar for gamma radiation. tungsten, and Ni with the majority of deletions being partial deletions.

There is currently no method to discriminate DU exposure from exposure to other genotoxins except for urine uranium analysis. The data suggest that HPRT mutation spectrum may be a potential biodosimeter for exposure to DU. In view of the current results and the known ability of DU to cause leukemia in rodents, further development of the HPRT assay as a biological dosimeter of DU and alpha particle exposure is warranted and could be beneficial in an attempt to discriminate between individuals exposed to DU and other genotoxic poisons.

Reconstruction of doses absorbed by radiotherapy patients by means of EPR dosimetry in tooth enamel

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Accuracy of doses delivered to patients undergoing radiotherapy is a crucial factor determining outcome of the treatment. According to commonly accepted ICRU recommendations, the difference between the prescribed and actually delivered dose should not exceed 5%. To assure such an accuracy limit individual planning of the radiotherapy treatment and verification of the actual doses by *in vivo* dosimetry are widely used in radiotherapy. However, when *in vivo* measurements are not performed for all fraction doses, verification of total, actual dose delivered during the treatment is impossible. This problem can be overcome by retrospective dosimetry based on reconstruction of the absorbed doses using EPR measurements of stable EPR signal induced in enamel by ionizing radiation. In this work we present a preliminary study aimed at comparison of the doses in tooth enamel calculated by radiotherapy treatment planning (RTP) algorithm (CadPlan 3.1) with doses reconstructed by EPR dosimetry. The study was performed for 8 radiotherapy patients treated by high-energy photon and electron beams from medical accelerators and ^{60}Co photons. In all cases the teeth were extracted within a few years after radiotherapy as a result of dental treatment. In 5 patients the examined teeth were irradiated directly by primary beam and the reconstructed doses were from 9.2 Gy up to 74.1 Gy; in 3 patients the examined teeth were beyond the irradiated field and dose to enamel due scattered radiation ranged from 0.59 Gy to 0.73 Gy. The precision of dose reconstruction was about 8%. The doses reconstructed in teeth positioned within irradiated field differed by -1% to +32% from doses calculated by RTP. For teeth positioned outside the primary beam the differences were up to 62%. Such a discrepancies between the calculated and reconstructed doses cannot be attributed to limited accuracy of EPR dosimetry and will be investigated in our further work.

EPR Investigation of Radiation Situation in Vicinity of Tailing Pool KOSHKAR-ATA.

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KOSHKAR-ATA is a closed lake/sedimentation tailing pool for industrial, toxic, radioactive (RW) and liquid domestic waste. It is situated 5 km north of Aktay in the Mangystau oblast at the Caspian Sea coast. Solid RW from a chemical-hydrometallurgical plant that processed uranium ore was trench-type buried without dampproofing, contrary to proper control and without proper accounting. According to data from the Mangystau oblast ecological authorities, 51.8 mln. tons of RW with 11,000 Ci total activity were placed in pool. The most hazardous elements in that waste are ^{238}U, ^{226}Ra and ^{230}Th.

Investigations of radio-environmental impacts usually study the distribution and migration of radionuclides. This approach, however, neglects issues related to the dose loads on the population in the vicinity of the tailing pool and potential negative changes in environment due to radiation.

This paper reports preliminary investigations of environmental samples and human teeth using EPR-dosimetry.

Based on EPR radiation signal in teeth enamel the total absorbed dose for several representative members of local population has been reconstructed. No high doses were revealed, but in several cases the observed radiation signal from teeth enamel clearly exceeds background values.

A high radiation signal was also found in various environmental objects such as soil, minerals, shells, etc.,. Generally speaking, paramagnetic centers that create those signals may be a considerable hazard for the population since they contain unpaired electrons with quite high chemical activity and, when inhaled with dust, for instance, may cause various pathologies including carcinogenic and mutagenic ones. This topical problem is still poorly studied and requires special consideration and thorough investigation.

EPR dosimetry for actual and suspected overexposures during radiotherapy treatment in Poland

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On February 27th 2001 five breast-cancer patients undergoing radiotherapy in the Bialystok Oncology Center, Poland, received a single, high dose of 8 MeV electrons generated by a Neptun 10p linear accelerator. The ultimate cause of the accident was a defective safety interlock and an obsolete safety system of the Neptun 10p accelerator leading to a large increase of the dose rate even though the display indicated a lower value than normal. The combination of these factors led to the substantially higher doses to the patients. All patients experienced immediate pain and skin reddening, followed by moist desquamation and development of deep necroses by autumn 2001. In spring/summer of 2002 all patients underwent a surgical reconstruction of the chest wall with subsequent skin transplantation. Two patients were treated in Paris, France and three in Kielce, Poland. In case of three patients pieces of rib bones were removed, allowing an estimation of the accident doses by electron paramagnetic resonance spectrometry (EPR) on the hard bone layer of each sample. EPR measurements were performed at the IRSN, France, and INCT, Poland.

The dose addition method was used by both teams to estimate the absorbed dose in bone samples. Post-irradiations were performed with a similar electron beam for IRSN and with ^{60}Co gamma-rays at INCT.

The values of the initial doses were composed of doses received by the samples during regular therapy before the accident and the dose from the accidental exposure. The former doses are known from the treatment histories and were subtracted from the obtained dose values. The doses delivered during the accident were as high as 60-80 Gy.

In 2005 a patient was treated for similarly deep necroses of the chest wall, which developed 6 years following a "standard upper mantel fields" therapy for Hodgkin's disease. The planned total dose was 32 Gy. In order to check the delivered dose, EPR dosimetry was performed on a bone sample taken from the first right rib. The results indicate that the received dose was in the range of 3-29 Gy showing that the necrosis is not due to an overexposure.

Combined EPR and TL measurements of Sr-90 contaminated teeth of Techa River residents

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Tooth enamel dosimetry is nowadays a widely applied method for reconstruction of past exposures to external high energy photon irradiation. In principle the method can be applied also to dose reconstruction following internal contamination by osteotropic radionuclides. These radioisotopes deposit in the tooth calcified tissues where they act as internal sources, so contributing to the dose in tooth. The interpretation of the EPR measurements in this case is complicated by several problems, one of which is the non-uniform distribution of the radioinduced free radicals, which reflects the inhomogeneous distribution of radioisotopes.

A TL and EPR combined method has been proposed for detecting the presence of internal radionuclides in whole teeth. A good correlation between the dose rate in TL and the volume averaged dose in enamel (determined by EPR) was observed, demonstrating that the method has potentials for assessment of internal dose induced by the current ^{90}Sr in dental tissues.

Aim of the present paper is to evaluate to what extent the EPR and TL measurements of a tooth (or of part of it) are representative of the average ^{90}Sr contamination to the tooth departments. This objective was achieved through investigation of in-tooth and inter-tooth variability of the EPR and TL measurements. The dose distribution in separate tooth departments (enamel, crown dentine and radical dentine) in ^{90}Sr contaminated teeth was investigated with the combined EPR and TL method. The measurements were performed for two cases of contaminated individuals resident in a village along the Techa River, in the South Urals, born at the time of the highest ^{90}Sr release from the Mayak plant in the late 40's. The teeth of these donors are expected to have incorporated a high concentration of radionuclide in both dentine and enamel, and therefore their external exposure can be assumed negligible. In particular:

a) the first donor was born in 1949 and donated 9 teeth. Measurement of these teeth is expected to provide information about the inter-tooth variability of internal dose;
b) the second donor was born in 1948 and donated one molar. Since this tooth was sufficiently big to allow separation in fractions of at least 50 mg, the EPR and TL measurements were performed on 10 fractions. These measurements were designed to provide information about the intra-tooth variability.

The obtained data show that the dose difference among various department of the tooth are larger than the uncertainty in the assessed dose and reflects in both TL and EPR measurements.

Exposure Subpopulations and Characteristics of the Individual Dose Distribution Among Inhabitants of the Semipalatinsk Region

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Issues surrounding the magnitude and distribution of radiation dose to inhabitants in the Semipalatinsk region still remain a challenge in spite of numerous investigations by several groups. At the 3rd Dosimetry Workshop on Hiroshima (Japan, March 2005), tooth enamel EPR dosimetry results from several groups together with data of a number of other methods were comparatively assessed and compiled. In general, the median dose among inhabitants of the Dolon and Sarjal, the most exposed from nuclear weapons test settlements, was estimated as 0.5 and 0.3 Gy, respectively. However, one may note, that at this analysis mainly EPR results on teeth sampled a rather long time ago were scrutinized.

In this presentation, we report on results of absorbed dose reconstruction by EPR dosimetry performed using teeth extracted in the period 2004-05 from inhabitants of the 3 highly exposed settlements Dolon, Sarjal, Mostik and the control settlement Maysk, for which both historical records and modern measurements suggest no radioactive tracks. Results indicate that each settlement had a standard distribution around a small dose. However, we did not expect to observe an extra more wide distribution in range more high dose, which was observed for all four settlements including Maysk, where there was no radioactive tracks and exposure levels were close to natural background.

We discuss possible reasons for observing the wide range in dose in these settlements, which is likely attributable to other routes of irradiation among exposure subpopulations in the studied settlements.

EPR and γ-spectrometeric researches of bottom sediments and soils in Syr-Darya uranium-ore province

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The river Syr-Darya is the basic lifeproviding fresh-water artery of Southern Kazakhstan. The intense epidemiological situation in this region has caused significant anxiety. Practically all medical parameters describing the state of health of local residents, including children, are essentially worse than the average for the republic. One of the most serious aspects of this problem is the weak level of scrutiny on the environmental influence of significant deposits (more than 15 % of world reserves) of natural uranium, and the consequences of technological and industrial work on its prospection, extraction and processing. The present work is devoted to studying by γ-spectrometry and EPR methods the radiation characteristics of some environmental objects in Syr-Darya river samples.

Research has shown the quantities of radionuclides (^{234}Th, ^{226}Ra, ^{214}Pb, ^{214}Bi, ^{210}Pb, ^{228}Ac, ^{224}Ra, ^{212}Pb, ^{212}Bi, ^{40}K and ^{137}Cs) in soil samples and bottom sediments selected at various points along the Syr-Darya river and tributaries Arys, Keles, Kurkeles. The results testify to the raised concentration of natural radionuclides, that, undoubtedly, is connected with the rather high contents of uranium and thorium in this region. The same samples have been studied by EPR method with purpose of detection and studying a size of a radiation signal.

The observed spectra have a complex shape, and show the presence of the specific paramagnetic centers of a radiation origin. Peculiarities of the EPR signal, a quantitative estimation of concentration of the centers of different types, and accuracy and reproducibility are considered.

The interrelation of EPR data and γ-spectrometer of researches, and also degree of correlation of indications of these methods depending on participation of the investigated samples in uranium deposits are discussed.

EPR tooth dosimetry of Semipalatinsk area inhabitants

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The accuracy of retrospective dosimetry for Semipalatinsk area inhabitants remains a topical problem in view of concern of local citizens in Kazakhstan about past exposures, ongoing epidemiologic studies, as well as our specific scientific interests. It is known [1-2] that absorbed doses attributed to the same settlements of this region obtained from different estimation techniques (i.e. EPR dosimetry using human tooth enamel, luminescent dosimetry using quartz extracted from bricks, and analytic dose reconstruction based on historical exposure rate measurements) may vary several fold. For example, absorbed doses assigned to the Dolon settlement resulting from different techniques lie in the approximate range 0.1-1 Gy though EPR tooth measurements are predominantly below 0.5 Gy. This large range highlights the need for additional measurements with high-precision dosimetric techniques and subsequent improvement to the calibration aspects of the calculation techniques.

This work details the reconstruction of absorbed doses for Semipalatinsk area inhabitants that were obtained using EPR dosimetry measurements conducted at NIST in two phases. The second phase of work, reported here in more detail, relied on methods developed at SCRM and successfully reproduced at NIST. The latter technique has been successfully tested in the course of several interlaboratory comparisons and may be characterized by the following attributes:

- any significant x-ray component of the measured dose due to dental practices is accounted for by comparing separate measurements of lingual and buccal parts of a tooth,
- the combined uncertainty (one sigma) for the determination of accident doses is approximately 30 mGy for doses lower 300 mGy, and approximately 10 % of the dose estimate for doses higher 300 mGy.

Doses of several tens of inhabitants from settlements Dolon, Sarzhal, Karaul as well as from Semipalatinsk city have been reconstructed using the described technique. Values of nuclear test (accident) doses were found to lie in the range from background level up to approximately 1 Gy, with one exception; the dose for one person from Semipalatinsk was approximately 8 Gy. The contribution of x-ray irradiation from dental practices to the measured dose was identified in a few teeth.

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The 1st Nuclear Test in the Former USSR of 29 August, 1949: Comparison of Individual Dose Estimates by ESR Retrospective Dosimetry with Calculation and Luminescence Retrospective Dosimetry Data for Dolon' Village, Kazakhstan.

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The village of Dolon' is located close to the radioactive trace from the 29.08.1949 nuclear test at the Semipalatinsk nuclear test site (SNTS).

The method of ESR dosimetry was applied to human tooth enamel samples to obtain retrospective estimates of cumulative external dose in the village. The results of measurements performed on 16 eligible tooth samples were used to validate the results of individual dose calculations. These ESR and computed dose values were then compared with the estimates of local dose in air obtained for four locations in Dolon' using retrospective luminescence dosimetry (RLD) with quartz inclusions extracted from brick samples taken from the local buildings.

The ESR dose values were compared with calculated individual external dose values for the same persons, individualized using special questionnaires designed to elicit information required to derive individual shielding and behavioral factors. Since previous calculations of external dose in Dolon' were based on the maximal dose rates along the axis of the trace (located NW of village), the local doses in the village were calculated in this study. This calculation was performed using published data for soil contamination in the vicinity and within Dolon' village by ^{137}Cs (137 samples) and $^{239+240}$Pu (76 samples); GPS data were available to fix the locations of each soil sample. Uncertainties in the calculated individual doses were estimated by performing Monte Carlo simulations.

The following ESR and computed individual dose ranges for inhabitants were obtained (here and further the uncertainties of 1 of average values are presented): 0 - 440 mGy (average 156±37 mGy) and 12-650 mGy (average 174±76 mGy) respectively. The doses in the air over whole village and for the southeastern part of the village containing the RLD sampling points were calculated to be 775±40 mGy and 645±70 mGy respectively. The latter compares well with the RLD estimate of 460±92 mGy. We suggest that an estimate of the "upper level" of the "shielding and behavior" factor of dose reduction for inhabitants of Dolon' village, of 0.28±0.07 can be obtained by comparing the individual ESR tooth enamel dose estimates with the calculated mean dose for the settlement.

Results of tooth enamel EPR dosimetry for population living in the vicinity of the Semipalatinsk Nuclear Test Site

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The method of electron paramagnetic resonance (EPR) dosimetry was applied to human tooth enamel to obtain individual absorbed doses of residents of settlements in the vicinity of the Nuclear Test Site (SNTS) in Semipalatinsk region, Kazakhstan. The distances between investigated settlements and Ground Zero (Site of surface and atmospheric tests) are in the range 70-200 km from SNTS. Tooth samples were extracted according to medical indications in the course of ordinary dental treatment. (In total, 117 tooth enamel samples were analyzed. 8 tooth samples were from control settlement Kokpekty, which were not subjected to any radioactive contamination and located 400 km to the Southeast from SNTS). According to the information obtained by questioning 3 tooth samples from Semipalatinsk City and 1 tooth sample from Charsk were subjected to x-ray examination of the jaw. Only molar teeth were used for dose determination.

It was found that the excess doses obtained after subtraction of the contribution of natural background radiation ranged up to about 450 mGy for residents of Dolon, whose tooth enamel was formed before 1949, and do not exceed 100 mGy for younger residents. For residents of Mostik, excess doses do not exceed 100 mGy for all ages except in one resident, for whom extremely high dose of 1250 mGy were registered. For Bodene settlement, excess doses higher than 100 mGy were obtained for two samples from the residents having enamel formed before 1949. An extremely high dose (2800±400 mGy) was obtained for one resident of Semipalatinsk City. This person spent a lot of time at the SNTS and such a high dose may be attributed to the effect of radiation, probably resulting from the nuclear tests. In general, the obtained results from Dolon are in agreement with the pattern of radioactive contamination of territory after the nuclear test of 1949.

Iodosimetry of two persons chronically exposed to Radium-226

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Two office workers were chronically exposed to external γ-radiation from a Radium-226 source which had been kept in a hospital safe for more than 30 years. One of the persons (A) had been working for several years in the same room where the source was located. The working place of the other exposed person (B) situated in an adjacent room and the time spent in this room was considerably shorter than for person A. Physical measurement of the source revealed a dose rate of 55 μSv/h inside the safe. Biological dosimetry was performed at STUK with both conventional dicentric and FISH methods in order to receive dose estimates of both short-term and long-term exposure. Routine 48-h whole blood cultures were set up, harvested and slides were prepared. Altogether 1000 metaphases of each case were scored from Giemsa stained slides. FISH was performed using biotinylated whole chromosome probes for chromosomes 1, 2 and 4 with FITC detection and propidium iodide counterstain. Scoring of FISH painted slides encompassed 4395 metaphases (i.e. 1560 genome equivalents, G.E.s) for person A and 3000 metaphases (i.e. 1100 G.E.s) for person B. For person A, the chronic dose based on dicentrics was estimated to 0.7 Gy (95% confidence interval 0.2 and 1.2 Gy), whereas the FISH analysis resulted in a dose of 0.6 Gy (C.I. 0.1 and 1.1) when the age-dependent control level of translocations was accounted for. For person B, no dose was received according to the dicentric analysis. The FISH analysis pointed to a dose of 0.4 Gy (C.I. 0.01 and 0.8 Gy). The results show large uncertainties in dose estimates which are to a great extent caused by uncertainty in the linear coefficient. The dose estimates will be discussed in light of the physical dose rate measurements.

Influence of Clonal Structure of Blood Lymphocytes in Retrospective Cytogenetic Dosimetry.

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Clonal chromosome aberrations were detected by scoring 5,000 T lymphocytes from each of five atomic bomb survivors. The observed translocation frequencies were 6% to 12% with FISH using probes for whole chromosomes 1, 2, and 4. After FISH screening of translocations involving the painted chromosomes, all the slides were re-stained for Q-banding to determine the unpainted, counterpart chromosomes and the breakpoints involved in the translocations to identify clonal aberrations (i.e., identical aberrations carried by at least three cells). The minimum clone size detected was 3/5,000 or 0.06%.

Among 2,444 aberrant cells observed, 44 different clones were detected. Specifically, we found 31 clones (clone size; 3-5 cells/5,000 or 0.06-0.11%), 5 clones (0.12-0.23%), 2 clones (0.24-0.47%), 2 clones (0.48-0.95%), one clone (0.96-1.91%), 2 clones (1.92-3.83%), and one clone (3.84-7.58%). The number of clones was inversely related to the clone size as we found previously by scoring 500 cells from about 500 survivors (detectable clone size $\geqq$0.6%).

The results extended the concept of mosaic structure of lymphocyte pool down to as small as 0.06%, and indicate that the clonal cells detected represent the tip of an iceberg; *i.e.*, there are large numbers of further smaller clones (<0.06%) that escaped the present detection limit. The results indicated that the clonal structure should also exist among apparently normal cells. This means that clonally derived normal cells decrease the estimated frequency of non-clonal translocations (i.e., indicator of radiation dose), by 5-10% in retrospective biodosimetry.

PCC -Ring Dose Effect Curves in Human Lymphocytes Exposed to Gamma and Neutron Irradiation.

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The Prematurely Condensed Chromosomes method combines the possibilities of the efficiently premature chromosome condensation induction by the okadaic acid or the Calyculin A with the simplicity of the Giemsa staining. This method overcomes the three major problems of the conventional biological dosimetry by dicentric analysis at high doses, (i) the lymphopenia due to cell death reducing the number of lymphocytes available; (ii) the radio-induced cell cycle arrests causing low mitotic index. (iii) the dicentrics saturation at high doses. Therefore the mitotic index is low and the number of cells available to have a statistically significant result based on at least 100 cells is very difficult to achieve at high dose.

In this paper, dose-effect curves for dose assessment for gamma and neutron high doses overexposures are presented. The dose range of the curves is from 5 Gy to 25 Gy.
For the elaboration of these curves 9 676 PCC cells in G1 G2 and M/A stages were analyzed. The results were fitted to a lineal quadratic model in gamma irradiation and showed saturation starting from 20 Gy. For neutron irradiation the data was fitted to a lineal quadratic model up to 10 Gy, and then a markedly cell cycle arrest and saturation was observed.

These curves are of particular interest for victims exposed to doses exceeding 5 Gy above which it is always very difficult to estimate a dose using the conventional technique.

Method for Efficient Establishment of Technical Biodosimetry Competence

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While the dicentric assay is the current gold standard in biological dosimetry, the method requires some degree of technical competence. This expertise is usually developed over time by the evaluation of many hundreds of metaphases. This competence is usually documented through establishment of a dose response curve, which is required by any laboratory performing cytogenetics for biological dosimetry purposes. However, competent and consistent evaluation of metaphases must be established for any new observer in a laboratory that should contribute to the analyses for biological dose assessments. Consistent evaluations within a laboratory are a necessity and discrepancies in scoring can seriously jeopardize the reliability of any assessment.

The Swedish Defence Research Agency (FOI) together with the Norwegian Radiation Protection Authority (NRPA) has conducted an inter-calibration exercise for the purpose of establishing comparable scoring criteria for evaluation of aberrations in metaphases. The exercise further revealed specific aberrations that were difficult to identify and were consistent sources of uncertainty. Another outcome of this exercise was the development of a method report detailing the lab's scoring criteria with numerous visual examples. The final outcome of this exercise was the development of a strategy for establishing technical competence in metaphase scoring in an efficient manner. The key components of the strategy in short are the review of guidance for biodosimetry methods, performance of an inter-calibration exercise with a previously established data set, review of incongruous evaluations with a well-established observer, a follow-up exercise depending on the outcome if the first exercise, and finally and inter-comparison to document the mandatory agreement within 20% (ISO 2004). The outcomes of the inter-calibration exercise and the strategy will be presented in more detail.

The methods suggested here could be applied for the training of new or additional personnel. Documentation of such methods in other laboratories and similar strategies for training could facilitate more consistent scoring criteria among the biodosimetry community, a problem observed in previous international inter-comparisons (Lloyd 1987, Roy 2004). Better consistency among biodosimetry laboratories could provide an opportunity to reliably share the work load among different members of the biodosimetry community in the event of a mass casualty accident.

Increasing Canadian biological dosimetry capacity through the Cytogenetic Emergency Network (CEN)

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In the event of a large scale radiological/nuclear emergency, biological dosimetry is an essential tool for providing timely assessment of radiation exposure for screening the general population and identifying first responders who must be restricted from further exposure. The frequency of radiation-induced dicentric and ring chromosomes, found in lymphocytes blocked in metaphase, can be converted to dose estimates using the dicentric assay. Traditionally up to 1000 metaphases per sample are analysed, allowing detection of exposures as low as 0.15 Gy. However, when turnaround time is critical, such as with a large number of samples following a radiation accident or terrorist attack, the detection threshold can be raised to 1 Gy for initial triage, thus reducing the number of metaphases to be analysed to 50. More metaphases can be analysed later to refine these dose estimates.

Currently, Canada has four core laboratories that can perform the dicentric assay for biological dosimetry. We are developing a network of clinical cytogenetic laboratories across Canada to increase this capacity for emergency situations. A workshop on biological dosimetry was held in May 2004 for interested clinical cytogenetic laboratories. Blinded slides, prepared for dicentric assay analysis following in vitro irradiation of blood from a healthy volunteer to a range of gamma-ray doses, were distributed to the participants and to the four core laboratories. A total of 41 people at 22 different laboratories analysed a minimum of 50 metaphases per slide to mimic triage scoring. Dose estimates were calculated based on a dose response curve generated at Health Canada. Of the 104 slides of 50 metaphases analysed, 92 (88.5%) of the resulting dose estimates fell within the expected range using triage scoring criteria established by Lloyd et al. (2000). In fall 2007, blood samples will be shipped to interested clinical cytogenetic laboratories that participated in the 2004 scoring exercise for assessment of culturing and slide making techniques and chromosome analysis. This network will increase our scoring capacity 20-fold and will be ready to respond to radiological/nuclear emergencies and provide triage quality biological dosimetry.

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First operational protocol for emergency dosimetry based on EPR in fingernails and hairs

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Most of the existing methods of biologically-based post-exposure assessments of radiation dose have three potential types of limitations: high labour intensity, low yield, and an intrinsic time lag (up to several days) because of the time required for the development of the radiation-induced biological effects that are assayed. These drawbacks reduce their usefulness for immediate triage. X-band EPR dosimetry with extracted teeth also has limited applications for this purpose because it requires extraction of the tooth. In contrast, finger and toenails or hair can be easily obtained from individuals immediately after potential exposures. We therefore are developing procedures for making such measurements. The potential practical imitations of this approach include fading of the radiation-induced signal (RIS), the presence of background signals, and signals induced by mechanical stress during sample collection. To deal with these potential problems sand to achieve the goal of rapid triage, we propose a two-stage procedure. In the first stage, a potentially exposed population can be rapidly triaged based on a rough dose assessment by EPR measurements in fingernails and hairs using standard averaged values of dosimetric characteristics of samples, i.e. fading constant, radiation sensitivity, and amplitude of the background signal. In the second stage the accuracy of the dose measurements can be markedly improved by longer measurements that permit the determination of the mentioned above dosimetric characteristics for each sample. Further, decay of the RIS decay can be considerably reduced by sample storage at low temperature (<0 °C). Based on our results to date we estimate that we can achieve detection limits of 1.8 Gy for fingernails and about 3 Gy for hairs. These levels should be achievable using only about 5 minutes for each measurement. This has the promise of providing rapid assessment of dose in a large number of individuals in a short period of time, which could be very useful for the initial triage of a potential mass-casualty radiation incident or accident. The presentation will review that data obtained so far and the basis of the extrapolation to the operating characterized described above.

Potential Prognostic Significance of Changes in Apoptosis-Related Pathways for Biodosimetry and Therapeutic Efficacies

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We have found that ionizing ^{60}Co gamma radiation injury produces temporal- and dose-dependent changes in apoptotic-related pathways in multiple human and animal models. The BAX gene regulates apoptosis in cellular pathways involving anti-apoptotic activities of BCL-2 and GADD45a. The ratio of BAX to BCL-2 expression determines survival or death following an apoptotic stimulus. On the other hand, cell proliferation capacity and cellular carcinogenesis are controlled by telomerase, the core components of telomerase being activation of human telomerase reverse transcriptase (hTERT) and constitutively-expressed human telomerase RNA (hTR).

In order to determine prognostic significance of these gene targets for biodosimetry and potential prognosis after radioprotector (genistein) and antibiotic (ciprofloxacin) treatments, we examined gene expression changes by multiplex QRT-PCR assays. Total RNA was isolated from whole blood at different times post-irradiation to a broad range of radiation doses. Studies were conducted in *ex vivo* irradiation models and *in vivo* in rodent, non-human primate, and radiotherapy patients undergoing total-body irradiation prior to bone-marrow transplantation.

Data from these models demonstrate dose-dependent increases in GADD45a, and decreases in BCL-2 and hTR expression, at different time points after irradiation. In genistein-treated human whole blood, concentration-dependent enhancement of GADD45a expression and changes in apoptotic regulation by BAX/BCL ratios were observed *ex vivo* at 24 h after a 2-Gy dose of gamma radiation.

Rhesus macaques were irradiated with 6.5 Gy ^{60}Co gamma radiation at 0.4 Gy/min total-body-irradiation. The macaques were administered six doses of oral ciprofloxacin (30 mg/kg) beginning on day 5 post-irradiation. On day 7, increased levels of GADD45a and BCL-2 mRNA were observed, demonstrating that antibiotic treatment affects gene targets associated with resistance to apoptosis.

We are currently investigating whether hTERT and hTR expression correlates with increased expression of BCL-2, which would imply a coupling between telomerase reactivation, proliferation, and resistance to apoptosis. Our data provide evidence that gene expression patterns will be useful in the assessment of radiation injuries, as well as in determination of potential therapeutic efficacies.

Inter-laboratory comparison of EPR dosimetry with tooth enamel in the SOUL project

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The main goal of the project Southern Urals Radiation Risk Research (SOUL) is the exploration and quantification of health risks due to chronic exposures to plutonium, strontium and external radiation. This will be done by improving, updating and analysing dosimetric and health data for the Mayak workers and Techa River cohorts. A key dosimetric element is electron paramagnetic resonance (EPR) measurements of tooth enamel which is aimed at quantifying external dose values, in order to validate or improve external dosimetry systems.

In the SOUL project dose estimates from EPR measurements of tooth enamel will be provided by three laboratories. In order to use these data for a joint analysis a unified quality assurance protocol has to be implemented and systematic deviations between the dose estimates have to be determined. The protocol will be established on basis of results that are presented in this paper from an inter-laboratory comparison performed between the EPR laboratories involved in the SOUL project.

Each laboratory prepared a pool of enamel powder, and divided it in 30 aliquots of 100 mg. Groups of 5 aliquots were irradiated in the own laboratory with dose of 0.1, 0.2, 0.5, 1.0 and 1.5 Gy, and one group of 5 aliquots was kept unirradiated. These samples were used as standard samples for laboratory sample preparation methodologies and transfer between laboratories for EPR measurements. Each laboratory measured the three standard sets. Laboratories evaluated for each set of standard samples detection limit; calibration uncertainty and parameters of the EPR-signal-to-absorbed-dose calibration curve. Effects of sample preparation, EPR signal evaluation and EPR equipment on quality of dose reconstruction will be analysed.

Dosimetry based on environmental objects

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In addition to the well known potential for the use of teeth and bones for after-the-fact dosimetry, it may be possible to use non-living material such as articles of clothing and other materials likely to be on the person or in the immediate environment at the time a radiation exposure occurs. There are several potential features of this approach of measuring environmental objects that could complement *in vivo* EPR tooth dosimetry and other dosimetry approaches:

- Greater sensitivity- higher frequency EPR spectrometers can be used because samples do not contain lossy materials that would cause non-resonant absorption. If local heating occurs, it would not be a concern in these non-living materials.
- Greater precision- because these are inanimate objects studied *in vitro*, it is possible to use the "radiation-added" method of dose calibration.
- Homogeneity of exposure - measurements would be made at a different site than *in vivo* measurements of teeth
- Effect of neutrons and gamma rays can be determined- many of the materials in the environment, unlike the teeth used for *in vivo* measurements, will respond to neutrons as well as gamma rays.

We have begun testing several categories of materials including clothing fabrics, shoes and belts, stationary and office supplies, building and structural materials, portable electronics (cell phones, etc), jewelry and watches, candy, credit cards and currency. These categories of materials were selected because of the high likelihood that they would be in the immediate vicinity of individuals who were potentially exposed in a radiological incident.

Our experimental approach involves obtaining background signals on unirradiated materials using a conventional (9.5 GHz) EPR spectrometer under the room conditions. If no EPR signal is present that is likely to interfere with the study of a radiation-induced signal, then the samples will be irradiated to 3000 cGy. After this high dose, if a potentially quantifiable EPR spectrum is observed, then these materials will be considered as potential candidate dosimeters. Fresh, unirradiated candidate dosimeters will then be irradiated at lower doses (100-1000 cGy) to obtain a quantitative and relevant dose-response relationship. We also will determine if there are any time dependent changes in the radiation-induced EPR signals.

Our current research focuses on determining the potential value of this approach. If it does appear to be valid and useful, considerable additional studies would be needed to convert it into a field deployable approach, but there do not seem to be any intrinsic barriers to making such a transition. It also would be possible to enhance sensitivity by making the field measurements at other frequencies. Determining absorbed radiation of objects on a person or in the immediate vicinity of a radiological incident therefore has the potential to provide useful and complementary information to *in vivo* EPR dosimetry measurements.

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Radiation Exposure Measurements for Military Participants in US Nuclear Weapons Tests Using EPR in Dental Enamel

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The criteria for ideal exposure measurements suitable for dose-response analysis in epidemiology for military participants in U.S. nuclear weapons tests ("Atomic Veterans"), as prescribed by the "Five Series Study" (Nat' Acad Press, 2000), are: (1) individual specific, (2) recorded by time, duration and dose, (3) sensitive to different radiation components of exposure, (4) previously validated for use in similar situations, (5) complete: cover all exposures for all people, and (7) accepted by all interested parties. An additional requirement can be adequate sensitivity (LLD).

This report will discuss how well EPR dosimetry in dental enamel satisfies these criteria. In addition, it will present a history of preliminary EPR measurements on enamel samples obtained from teeth discarded in the normal course of dental treatment of the so-called Atomic Veterans. Difficulties in dose calculations and interpretation of the EPR data will also be covered. In particular, problems associated with separation of the doses due to exposure to high-energy gamma-rays from the dose due to medical diagnostic x-rays will be reviewed.

Dental enamel maintains a record of a tooth's exposure to gamma- and x- radiation. The absorbed dose is stored in the form of long-lived free radicals that can be detected using Electron Paramagnetic Resonance (EPR). Aldrich and Pass in 1986[1] developed a technique using EPR for separating the contributions from diagnostic and high energy radiation to total exposure. They then applied this technique to enamel obtained from discarded teeth of Atomic Veterans. Preliminary results published in 1989[2] indicated exposures to medical diagnostic radiation may be significantly higher in this cohort as compared to the general population. This study was, however, limited by, among other factors, the low sensitivity of the Varian EPR spectrometer used at that time.

The veterans enamel samples were measured again in 1999-2000 using a state-of-the art Bruker EPR spectrometer. This report will review the results of the 1989 study and the analyses currently underway of the newest data. The problems encountered with both data sets and, consequently, the suitability of EPR in dental enamel for epidemiologic studies of the veterans cohort will be then be addressed.

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Applicability of EPR dosimetry with teeth to dosimetric support of epidemiological studies: practical aspects

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EPR dosimetry with tooth enamel proved to be precise and sensitive method of retrospective dosimetry with properties superior to majority of other dose reconstruction techniques. However, practical application of EPR dosimetry, in particular, its utility for dosimetric support of radiation effects epidemiological studies has some peculiarities and is endowed with certain limitations. The paper is based on the experience, which was accumulated in course of regular application of EPR dosimetry with teeth in the framework of large scale cohort and case-control studies among Chernobyl liquidators and is dealing with discussion of the practical aspects of its utility.

One of the implicit problems for broad application of EPR dosimetry is a limited availability of teeth. This limitation could be addressed by elaboration of a widespread tooth acquisition network, which should operate in passive mode, e.g. by collection and registration of teeth extracted in course of normal dental practice. Such network had been established in Ukraine and in course of its operation about 7,500 teeth from Chernobyl clean-up workers were collected covering thus about 5% of the most exposed population.

Other fundamental limitation is related to the fact that EPR dosimetry with teeth *per se* is capable of measurement of cumulative dose only, which, in general, includes several components - natural radiation, medical and occupational exposure, dose of interest, i.e. due to radiation accident. Significantly, confounding factors in EPR dosimetry add unknown dose to the quantity of interest, e.g. if not properly accounted for, they could lead to overestimation of the dose of interest, sometimes substantial.

The most significant confounders are caused by UV exposure (which affects front teeth only and can be avoided by exclusion of incisors and canines from consideration) and medical x-ray procedures, both diagnostic and therapeutic. The problem of dental x-ray examinations can be addressed by analysis of dose profiles in teeth, while other medical applications do not make visible signature in dose profiles and thus possible contribution of these procedures can be accounted by close investigation of personal radiation history of a potential study subject. The results of validation studies support this point and will be discussed in the presentation.

In total, despite existence of pitfalls in EPR dosimetry with teeth, general balance is positive, given proper account and mitigation of confounding factors associated with this technique. Ignoring the effect of these factors may lead to completely misleading results, deteriorating thus the value of EPR dosimetry as a powerful tool for dose reconstruction.

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Radiological Emergency Response Dosimetry in the United States

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At present the federal emergency response organizations in the US do not have retrospective dosimetry capabilities formally established. All dose assessments would be based strictly on modeling and calculations. Historical intentions for this work were that calculated values be verified using cytogenetic techniques through the Radiation Emergency Assistance Center/Training Site. This capability is presently being brought back through various funding agencies but is not expected to be operational until late in 2006.

Similarly, solid state techniques for biodosimetry are not formally integrated into the Federal Radiological Monitoring and Assessment Center (the emergency response organization for the federal government). No specific plans are in place for conducting any dose reconstructions using electron paramagnetic resonance of biomaterials, or thermoluminescence or optically stimulated luminescence of tiles, bricks or similar dosimetric materials.

Potential Scenarios of Terrorist Attacks and Radiation Accidents: The Need for Retrospective Biological Dosimetry of Acute Radiation Overexposures

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Around four hundred acute radiation overexposures have been formally reported, 134 of them just as result of the Chernobyl accident. Probably, this figure is not representative of the large number of overexposures that most likely have actually occurred, both in the earlier times when little was known about radiation effects and also more recently in countries with weak radiation safety infrastructures. Many overexposures are simply unnoticed, misdiagnosed, untreated and obviously unrecorded. Acute overexposures are, therefore, not low probability events: they have actually happened and, unfortunately, they will continue to arise as the use of radiation expands. Accidents with radiation sources have taken place at a rate of several per annum and they will perhaps increase in the future, as radiation is becoming a fashionable tool of medical diagnosis and treatment. Severe nuclear accidents, such as Chernobyl, are improbable but they cannot be discounted (international legally binding undertakings commit parties to be ready for that remote eventuality). Speculations of 'nuclear' terrorist attacks have increased after the devastating events of September 11th, and the possible scenarios include the explosion of 'improvised nuclear devices', sabotage of nuclear installations, and the malevolent use of radiation sources containing radioactive materials.

The paper will review all these possible situations of acute overexposures vis-à-vis the potential of what is termed 'biological dosimetry'. Although the use of the term has sometime been constrained to radiation-induced chromosome aberration analyses, it is widely intended to mean the retrospective assessment of radiation dose incurred by an individual through analyses of various kinds, including general radiopathology, hematology, chromosomal studies and electron paramagnetic resonance (EPR) measurements. The paper will claim that, while physical dosimetry is a necessary instrument for planning *a priori* radiation protection, retrospective biological dosimetry is the essential decision-aiding tool in the aftermath of the all potential scenarios of overexposure situations.

The paper concludes with a petition to competent authorities to follow international commitments and recommendations on radiation emergency planning by enhancing and strengthening national capabilities on biological dosimetry.

Medical Treatment of Radiation Injuries—Current U.S. Status

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A nuclear incident or major radioactive materials release would likely result in vast numbers of patients, many of whom would require novel therapy. Although the number of radiation victims in the United States (USA) has been limited, we base our doctrine for treating radiation injuries on i) historical data, ii) animal research, and iii) human results derived from present medical treatment such as the care provided to cancer patients undergoing radiation therapy or chemotherapy.

The medical management of radiation injury is complex. Radiation injury may include acute radiation sickness (ARS) from external and/or internal radiation exposure, internal organ damage from incorporated radioactive isotopes, and cutaneous injury. Human and animal data have shown that optimal medical care may nearly double the survivable dose of ionizing radiation. The currently recommended treatment of ARS in the USA involves supportive care, early use of leukopoietic cytokines, and judicious use of antibiotics. Supportive care for any significant radiation injury would also require the use of intravenous fluids, antiemetics, antidiarrheals, pain medications, and blood product support. The role for cytokines will be described in some detail. The role for stem cell transplantation is controversial as no survival benefit has been demonstrated for this modality.

The U.S. Food and Drug Administration (FDA) has published clear guidelines for the use of potassium iodide (KI) to block uptake of radioactive iodine. Treatment of internal contamination requires identification of the radioactive isotope and then appropriate individual programs of treatment. The FDA has approved the use of diethylenetriaminepentaacetate (DTPA) for the treatment of internal contamination with plutonium, americium and curium. The FDA has also approved the use of ferric ferrocyanide (Prussian blue) for the treatment of internal contamination by radioactive cesium or thallium.

Cutaneous radiation injury therapy may be quite protracted and require the expertise of reconstructive surgeons and other specialists. While significant advances in treatment of radiation injury have been made over the past 15 years, near-term novel therapies appear to offer excellent prognosis for radiation casualties. If a mass-casualty situation occurs, there will be need to perform rapid, accurate dosimetry and to provide medications to ameliorate radiation injury. Treatment of both the acute deterministic effects of radiation injury and the long-term stochastic sequelae of radiation damage are areas ripe for research.

Overview of *In Vivo* EPR, Including Use in Human Subjects

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The development and use of *in vivo* techniques for experimental applications in animals has been very successful and continue to grow significantly. These results have made possible some very attractive potential clinical applications as well. This presentation will provide an overview of recent developments, with an emphasis on those that are extending into use in human subjects.

The area with the most obvious immediate, effective, and widespread experimental and clinical use is oximetry, where EPR almost uniquely can make repeated and accurate measurements of pO_2 in tissues. Such measurements can provide clinicians with information that can impact directly on diagnosis and therapy, especially for oncology, peripheral vascular disease and wound healing. The other area of immediate and timely importance in human subjects is the unique ability of *in vivo* EPR to measure clinically significant exposures to ionizing radiation 'after-the-fact', such as may occur due to accidents, terrorism or nuclear war.

There are a number of other capabilities of *in vivo* EPR that also potentially could become extensively used in both experimental animals and human subjects. In pharmacology the unique capabilities of *in vivo* EPR to detect and characterize free radicals could be applied to measure free radical intermediates from drugs and oxidative process. A closely related area of potential widespread applications is the use of EPR to measure nitric oxide. These often unique capabilities, combined with the sensitivity of EPR spectra to the immediate environment (e.g. pH, molecular motion, charge), already have resulted in some very productive applications in animals and these are likely to expand substantially in the near future. They should provide a continually developing base for extending clinical uses of *in vivo* EPR. The challenges for achieving full implementation include adapting the spectrometer for safe and comfortable measurements in human subjects, achieving sufficient sensitivity for measurements at the sites of the pathophysiological processes that are being measured, and establishing a consensus on the clinical value of the measurements.

Electron Paramagnetic Resonance radiation dosimetry with fingernails

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There have been only a modest number of previous studies of radiation-induced signals in fingernails and while there were some promising aspects, overall the results were inconsistent and had some aspects that indicated that they would be unlikely to be useful for dosimetry. These including an indication that the signals decreased rapidly with time after 24 hours and that the lowest dose that could be estimated was several Gy. But there now is an increased need for after-the fact dosimetry due to the increased risk of clinically significant exposures due to terrorism or accidents. In particular there is a need for measurements that can be made in individuals rapidly and with sufficient accuracy to enable effective triage to be carried out. Using the capabilities of new EPR instrumentation and the experience gained in the use of teeth for after-the-fact dosimetry, we have undertaken a systematic investigation of the potential use of fingernails (and presumably toenails as well) as dosimeters that could be used in potentially exposed populations to determine if they have received exposure to radiation doses that could be life threatening. Our studies include measurements of the dose and energy dependence of radiation-induced signal (RIS), the rate of decay of the signal, variations between individuals in the RIS produced at the same dose, and the stability of the RIS signal during storage at different temperatures. The signal intensity was found to be linear with the absorbed dose to at least 50 Gy. The RIS signal did fade significantly by one week but was sufficiently stable to be readily measured with 24 to 48 hours, which is the period in which the method is most likely to be needed. Our current estimate of the lowest detectable dose with useful accuracy is about 2 Gy without any specific sample preparation. There are a number of potential methods to improve this limit significantly and to extend the time interval over which measurements can be made accurately. These studies are in the process of being carried out. But even at the present state of development, it seems quite possible that it will be feasible to use fingernails as an indicator of the severity of exposure in individuals. This may be especially effective as a complement to other techniques such as measurements of dose from teeth in situ.

Statistical Methods for Dose Reconstruction Using *in Vivo* EPR Tooth Dosimetry

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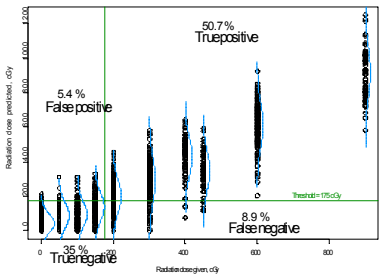
The signal obtained with in vivo EPR has an intrinsic stochastic nature and involves multiple sources of variation, such as the positioning of the resonator, geometry of the tooth, amount of enamel, and effects due to the soft tissues in the immediate environment pf the tooth. Although much effort has reduced the impact of these sources the dose prediction still possesses substantial variability. Thus, statistical methods are important tools for a sophisticated estimate of the radiation dose.

Adjusting for the tooth size. The tooth size affects the signal amplitude. We have found that the tooth surface, computed as the product of the lengths in two perpendicular directions, improves the calibration curve. In particular, we suggest the following calibration curve adjusted for the tooth size: A=a+b*(R*S), where A is the peak-to-peak amplitude, R is the radiation dose, S is the tooth surface, a and b are the estimated parameters. The interpretation of the parameters is that a is the amplitude associated with the background signal and b estimates the amplitude increase due to the dose of radiation per unit enamel surface. Incorporation of the tooth size in the calibration curve leads to up to 20% reduction of the uncertainty in the dose prediction.

Standard error of the dose prediction. Once a calibration curve is obtained, we estimate the dose as D=(A-a)/(bS). If parameters a and b were estimated precisely, the only error would be associated with measuring individual amplitude A. However, due to various sources of variation mentioned above, the calibration curve is not precise, so that we need to take into consideration three other parameters, var(a), var(b) and their correlation (these parameters are obtained from linear regression fitting). To evaluate the error of the dose prediction we employ the technique called inverse regression (Draper and Smith, 1998). We developed formulas for calculation of the dose prediction that reflects variation in A, a and b.



Sensitivity and specificity. Instead of continuous dose prediction one may predict the probability to be exposed to a specified level of radiation. The associated errors are known to be False negative and False positive. For example, as follows from the figure, the probability that the reverse is true when there is a prediction that a tooth received a dose larger than 175 cGy, is 5.4%. The advantage of this approach is that it does not require specification of the calibration curve. Furthermore, the ROC curve can be derived and used for the analysis of sensitivity and specificity of the EPR dosimetry at various thresholds.

Experimental Procedures for Sensitive and Reproducible *In Situ* EPR Tooth Dosimetry

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The ability to perform EPR radiation dosimetry using intact teeth *in situ* would extend this usefulness of the technique, especially for widespread use with the general population following an accidental or deliberate release of radiation [1]. There are several important technical and practical challenges that must be addressed to facilitate such measurements in the mouths of human subjects. Based on existing facilities (a permanent magnet capable of accommodating a human subject, accompanying *L*-band spectrometer and external-loop resonators), we have developed techniques to address these challenges and provide rapid and reproducible high sensitivity measurements in the mouths of human subjects.

Reproducible placement of the EPR resonator on the surface of the interrogated tooth has been a major focus of our efforts. We have addressed this challenge through the use of external loop resonators with detection loops designed to closely match tooth surfaces and development of precise and robust procedures to position and fix these resonators. Dentistry techniques and equipment have been identified to provide aseptic oral access for the resonator, to enable adequate visual inspection of the position of the resonator, and to comfortably stabilize the jaw during measurements. These steps also act to keep the presence of lossy tissue and saliva from adversely affecting the sensitivity and variability of the dose measurement. A patient bed and chair have been designed to comfortably position and immobilize the patient in the magnet.

The EPR signal intensity is affected by a number of instrumental parameters, including the modulation amplitude and B_1 magnetic field amplitude at the tooth. Temporal instability or spatial inhomogeneity in these parameters can reduce the precision of the estimated radiation dose. We have developed specially mounted reference standards that allow these parameters and the general performance characteristics of the spectrometer to be measured prior to and during the dosimetry measurements. The information from these standard measurements is incorporated into the data analysis to reduce variability in the dose estimates.

In vivo and supporting *in vitro* measurements will be presented that demonstrate the ability of these techniques to enable reliable *in situ* measurements of the radiation dose dependent signal in tooth enamel.

[1] Swartz, H.M., Iwasaki, A., Walczak, T., Demidenko, E., Salikov, I., Lesniewski, P., Starewicz, P., Schauer, D. and Romanyukha, A. Measurements of Clinically SignificantDoses of Ionizing Radiation using Non-invasive In Vivo EPR Spectroscopy of Teeth In Situ. Appl Radiat Isot. 62(2): p. 293-9. (2005)

Implementing EPR Dosimetry for life threatening Incidents: factors beyond technical performance

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The purpose of this paper is to present a framework for thinking systematically about what must occur if EPR is to become a widely available tool for emergency dosimetry. Typically, this translational research process for a technology as complex as EPR takes on the order of 10-20 years, but the current stage of development of EPR and the urgent nature of the problem it aims to solve makes it reasonable to expect that EPR could become a "work-in-progress" tool within a year and have a commercially produced system within 2-3 years if development proceeds systematically.

The capability that EPR dosimetry provides is triage: classifying patients as not needing acute treatment, likely to be responsive to treatment, or likely to be unresponsive to treatment. The requirements to make these classifications usefully includes a minimum sensitivity of 200 cGy, with increasing usefulness as sensitivity increases to 50 cGy; ability to be deployed in the field; and a processing time per subject of less than 15 minutes by minimally trained technicians.

Assuming that the technical goals are met or exceeded, the following steps need to be accomplished to achieve the goal of implementing EPR dosimetry:

1. Development of a field deployable version that is sufficiently robust, simple to operate, and provides an immediate and unambiguous output
2. FDA approval. The FDA has recognized the need to speed access to technologies such as EPR dosimetry that aid in homeland security, while managing its statutory requirements that only "safe and effective" devices are approved. Therefore, it can be reasonably expected that regulatory approval will be challenging, but not as daunting as might be expected for a similarly complex technology without applications to homeland security.
3. Acceptance by key decision makers in the federal government that the technology is a desirable approach for implementation
4. Acceptance by the actual users for use under realistic conditions
5. Commitment of commercial entities to the production of the dosimeters. To efficiently translate EPR into the marketplace, commercial investment or strategic partnership with a company that has expertise in the manufacture and/or distribution of such a technology will almost certainly be required. We are unlikely to obtain firm commitments only after we have demonstrated feasibility in suitable human subjects. The potential for other uses of in vivo EPR would significantly expand the potential customer base and thus attractiveness to a private investor seeking an adequate return on investment for technical development in EPR.

Mechanically-induced signal in fingernails as a confounding factor for EPR dosimetry

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By using EPR measurements of radiation induced radicals it is possible to utilize human fingernails to estimate radiation dose after-the-fact. This could be very useful under circumstances such as a radiological accident or terrorist event, to assist with rapid and effective triage of a potentially exposed population. One of the principal difficulties in this approach is the potential presence of artifacts due to mechanically-induced EPR signals (MIS) caused by mechanical stress during the collection and preparation of the samples. The MIS have spectral parameters (shape, g-factor and linewidth), which are very similar to the radiation-induced signal (RIS) and therefore, if not taken into account properly, could result in a considerable overestimation of the dose. We therefore have undertaken an extensive study of the origin and properties of the MIS so that we can develop reliable methods to separate the MIS separation from the RIS. The studies include the changes in intensity with time, the dependence on the size of the particles in the sample and the influence of the presence of oxygen when the samples of fingernails are obtained and prepared for study with EPR. We also have investigated the use different treatments with chemical reagents and heating. These studies are in process but the initial results indicate that we will be able to significantly reduce and perhaps eliminate the contribution of the MIS to dose estimate from the RIS.

EPR Dosimetry for Radiation Emergency in WHO-REMPAN Collaborating Centers and Liaison Institutions

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The WHO activities on strengthening preparedness and the system of response to radiation emergencies relies on its Radiation Emergency Medical Preparedness and Response Network (REMPAN) of medical and research institutions. The WHO-REMPAN comprises 32 institutions around the world providing unique expertise in the field of radiation emergency medicine, dosimetry, long-term treatment, follow-up, epidemiology, and public health.

Dose estimation at radiation emergency is a key factor for accurate prognosis for the life and the outcomes of the medical treatment for radiation exposed victims. Personal radiation dosimeters and cytogenetic analysis are most common dose estimation methods along with mathematical dose reconstruction. Methods of dose reconstruction have been intensively studied and further developed during the past decade. Often, retrospective individual doses assessments below the level of 100 mSv are needed. Personal dosimeters are not always available, chromosome aberration method is time-consuming, and the dose reconstruction is a subject to a great uncertainty. While, the EPR dosimetry could eventually become a method of choice, especially with the development of the *in-vivo* technique and soon to be available safe, quick, and reliable portable EPR tools that could be applicable for the prompt dose estimation in radiological accidents and nuclear mass-casualty events.

To identify the potential of the WHO-REMPAN in terms of providing emergency EPR dosimetry services and explore the opportunities for future collaborative and inter-comparison studies, a mini-survey was carried out. The survey focused on identification of capabilities, equipment, man-power, on-going research and willingness to collaborate in future inter-network projects.

The survey response rate was 69%. Eleven laboratories of the REMPAN centers are equipped and conduct EPR dosimetry using in vitro analysis on teeth, bones, nails, hair, alanine, sugar and cloth samples, using mainly *Bruker* and JEOL made machines. Among them, three labs are carrying the in-vivo EPR research using teeth and finger bones. The results of the survey demonstrated most of the responders are interested in collaborative research on EPR dosimetry. There is a need for establishment of international EPR network with several reference centers. Such cooperative effort will enhance the progress of this unique dosimetry method, which may eventually become of use in emergency situations. The WHO-REMPAN provides an international platform for fostering such collaboration.

Chromosome Network for Biodosimetry in Japan

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The present Chromosome Network in Japan was organized in 2001 just after Tokai-mura criticality accident for: 1) Integrating biodosimetry work to be ready for a large-scaled radiation accident, 2) Establishing a standard method for cytogenetical dose estimation, 3) Creating a standard dose response curve in Japan, 4) Preparing a training program for cytogenetical dose estimation, and 5) Training successors to be in biodosimetry work.

It consists of 7 laboratories in the following 6 organizations, i.e., National Institute of Radiological Sciences in Chiba, Asahikawa Medical College in Hokkaido, Institute for Environmental Sciences in Aomori, Aichi Human Service Center in Aichi, Osaka Prefecture University in Osaka, and Radiation Effect Research Foundation in Hiroshima. Those laboratories have the same systems such as an automated microscope called Metaphase Finder, a CCD camera system of microscope and an air-drying device named HANABI. Inter-laboratory communication is done via Internet.

One of our recent activities is to learn 1) the difference in selecting an analyzable metaphase and in scoring chromosome aberrations among examiners, and 2) whether those differences are permissible or not from the viewpoint of dose estimation. A chromosome preparation was made at NIRS using irradiated human lymphocytes at 1.0 Gy of ^{60}Co gamma ray according to the NIRS standard method. The 470 images automatically detected by the Metaphase Finder were numbered from 1 to 470, photographed with CCD camera under high magnification (1000x) and stored in a hard disk. Those 470 images were distributed to the said 7 examiners.

Each examiner selected 200 metaphases from those 470 images. The numbers of images screened for selecting 200 metaphases by 7 examiners were 269, 278, 293, 298, 323, 384 and 414, respectively. Numbers of dicentrics and rings accompanied by a fragment were 16, 21, 16, 19, 21, 20 and 24, respectively. Except for the last one, the estimated dose was within the confidence limits for 1.0 Gy by scoring 200 metaphases. Agreement rate of each metaphase was examined in 259 images. The metaphases selected by 7, 6, 5, 4, 3, 2, 1, and 0 examiner(s) were 117 (45.2%), 36 (13.9%), 19 (7.3%), 10 (3.9%), 10 (3.9%), 14(5.4%), 17 (6.6%), and 36 (13.9%), respectively.

Canadian Biodosimetry Capacity

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In December 2001, Canada's response to the international political climate was launched by the creation of the Chemical, Biological, Radiological and Nuclear (CBRN) Research and Technology Initiative (CRTI). The National Biological Dosimetry Response Plan (NBDRP), established through partnering the research efforts of three federal departments and one university, was created in response to this initiative. The NBDRP objectives were to develop a network of laboratories with expertise to perform biological dosimetry by cytogenetics and to investigate new technologies that may be applicable in the development of the new biodosimetry program.

Since the creation of the NBDRP, Canada has made significant progress in enhancing expertise and resources to be better prepared for Radiological/Nuclear events. Through participation in exercises, the existing capacities were tested and recommendations for improvements were made. Future exercises will further challenge the network and demonstrate the improved response capabilities; the next one is scheduled for March 2006. Progress made to the development of the Canadian Biological Dosimetry Program and some of the challenges encountered will be discussed.

Participation in exercises has promoted awareness of the NBDRP in the medical community resulting in the development of multidisciplinary networks aimed at improving Canadian Radiological/Nuclear emergency response and casualty management. Some preliminary ideas and results stemming from these networks will be discussed. (Funded by CRTI Project #0027RD.)

Biodosimetry Inter-comparison: FOI and DRDC Ottawa

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The biological assessment of radiation dose in individuals may be used to assess radiation exposure after an accident or in cases where an over-exposure is suspected and physical dosimetry is absent or uncertain. A reliable estimate of dose is critical for making life-saving medical decisions, assessing the long-term health consequences, and for reassuring persons with non-significant exposures. The dicentric assay, the current gold standard in biological dosimetry, requires some degree of technical capability and recently published ISO guidelines indicate the need for documenting competence and establishment of quality control programs. Intra- and inter-laboratory comparisons are required in order to document the ability to perform reproducible and accurate assessments.

The biodosimetry laboratories at the Swedish Defence Research Agency (FOI) and Defence R&D Canada (DRDC) Ottawa have conducted a concise inter-comparison for quality assurance purposes. The exercise involved the exchange of 3 previously prepared slides from each laboratory from samples that had been evaluated for each lab's dose response curve, to provide a total of 6 comparisons. Approximately 100 cells from each slide received were evaluated and aberrations frequencies reported and compared to the expected frequencies from each laboratory.

The result of the inter-comparison indicated remarkable agreement in aberration frequencies in all of the six samples. Comparison of dose estimates was not possible since FOI and DRDC Ottawa have established different dose response curves, for gamma radiation and x-rays, respectively. However, comparison of aberration frequencies, rather than dose estimates, illustrates highly consistent scoring criteria between the two laboratories. Another difference between laboratories was the evaluation and inclusion of centric rings by DRDC and evaluation, but not inclusion of these aberrations by FOI. Inclusion of rings for the purpose of this exercise did not present a problem. One final point addressed in this exercise was the evaluation of a slide with a large number of uncertain cells. A conservative approach where most cells were marked non-scoreable was compared to a more aggressive approach where the best judgment was given for as many cells as possible. Our comparison consistently indicated underestimate of aberrations with the conservative approach.

The exercise conducted by FOI and DRDC Ottawa provided an efficient means of documenting expertise. Such cooperation further establishes the international biodosimetry network and ensures our readiness for emergency response.

Building Connecticut's Clinical Laboratory Surge Capacity to Mitigate the Health Consequences of Radiological and Nuclear Disasters

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Introduction: Biodosimetry, based on the analysis of dicentric chromosomes in circulating lymphocytes, is considered the "gold standard" for estimating radiation injury, and is used to make informed decisions regarding the medical management of irradiated persons.

Objective: This abstract describes the development of biodosimetry laboratory surge capacity for the health consequences of radiological and nuclear disasters in Connecticut, including: (1) establishment of the Biodosimetry Laboratory for the timely assessment of radiation dosage in biodosimetry specimens; (2) identification of clinical laboratories qualified and willing to process biodosimetry specimens from a large number of victims; (3) training of clinical laboratorians in initial biodosimetry specimen processing; and (4) conducting a functional drill that evaluated the effectiveness of these elements.

Methods: Descriptive information was obtained from: (1) personal observations; (2) a needs assessment of clinical laboratories in Connecticut; (3) records from a training program of clinical laboratorians in biodosimetry specimen processing that was developed and provided by the Yale New Haven Center for Emergency Preparedness and Disaster Response; and (4) records from a statewide functional drill in biodosimetry specimen processing that was developed and conducted by the State of Connecticut Biodosimetry Laboratory.

Results: A Biodosimetry Laboratory was established at Bridgeport Hospital in a collaborative program between the Yale New Haven Center for Emergency Preparedness and Disaster Response, and the Connecticut Department of Public Health. A needs assessment of clinical laboratories in Connecticut identified 30 of 32 clinical laboratories qualified and willing to perform initial biodosimetry specimen processing. Currently, 79 clinical laboratorians in 19 of these qualified clinical laboratories have been trained in biodosimetry specimen processing. A functional drill was conducted, involving 37 of these trained clinical laboratorians in 18 qualified laboratories, as well as the Biodosimetry Laboratory. The average turn around time for biodosimetry specimen processing in this drill was 199 minutes. Drill participants provided feedback which will be used to further optimize biodosimetry specimen processing protocols in Connecticut.

Conclusion: Substantial progress has been made in the development of the necessary elements of clinical laboratory surge capacity for radiological and nuclear disasters in Connecticut.

Optimization Of Cytogenetic Procedures For Population Triage In Case Of Radiological Emergency

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In case of accidental overexposure to ionizing radiation, the scoring of dicentrics in lymphocytes from blood is the current reference method to estimate the dose received. When only few individuals are accidentally overexposed, at least 500 cells have to be scored to have a good estimation of the dose. But such a practice is too time consuming when many people are exposed such as in radiological emergency. In order to reduce the time required to estimate a dose, specific strategies were developed in the laboratory.

Population triage based on the adaptation of the dicentrics assay: in order to reduce the analysis time it is possible to have a dose estimation based on only 50 cells analyzed in an hour. Then the 95% confidence interval of the dose is 1Gy. This strategy was tested in the laboratory and give reasonable good results. However, in order to be ready to deal with many samples real time exercises are required.

Population triage based on the use of image analysis systems: in order to reduce the time required for analysis, image analysis systems can be used. One application of theses systems is the metaphase finder alone which increases the speed of the scoring by a factor 2. An other application allows the automatic detection of dicentrics. The system proposes to the operator some candidate dicentrics which are verified manually. In this case, fifty percent of the dicentrics are correctly detected. For 300 cells analyzed in half an hour, the 95% confidence limit of the dose found is 0.4Gy.

Population triage based on micronuclei assay: Even if micronuclei are less specific than dicentrics for radiation exposure at lowest doses, micronuclei are easier to score than dicentrics. It is then possible to estimate a dose based on the scoring of 500 binucleated cells in an hour with a dose confidence limit of 0.5Gy.
This assay has been tested in crises situations in comparison to the dicentrics assay. Thirty five samples were irradiated with doses from 0.5 to 3 Gy and analyzed by both techniques. In doses below 1 Gy the dicentric assay is more precise than the micronuclei assay whereas for high doses the opposite is observed.

Establishment of a network: biological dosimetry laboratories are of small size hence, their capacity can be overloaded by a large number of suspected overexposed individuals. That is why international and national networks should be established. To be operational such network should perform intecomparisons and population triage exercises.

Quantitative Expression of p53 and STAT3 Dependent Genes in Relevant Models for Biodosimetry Applications

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Ionizing radiation (IR) injury produces temporal- and dose-dependent changes in gene expression patterns in multiple human and animal models. Several downstream genes to the transcription factor p53 are up-regulated by IR, including *CDKN1a (p21$^{Waf-1/Cip1}$), GADD45a, DDB2*, and *BAX*. Intracellular stress-signaling for up-regulation of *CDKN1a* and *BCL-2* via the *STAT3* pathway has also been identified after IR injury and, together with p53 pathways, altered expression of these genes modify pathways involved in growth arrest, DNA damage response and repair, and apoptosis. In order to determine the prognostic utility of these gene targets for biodosimetry applications, we examined mRNA levels using *in vivo* and *ex vivo* radiation models. The objectives of this study were to (1) investigate the relationship between baseline mRNA expression levels in normal individuals; (2) define expression and patterns in response to IR; and (3) determine the inter-relationship of these genes. In addition QRT-PCR assays were developed, optimized, and validated for each model; proteins were examined by ELISA.

We irradiated whole-blood *ex vivo* to measure gene expression changes in samples from (i) a cohort of three healthy donors over a broad dose range (0, 0.25, 0.50, 0.75, 1, 2, 3 Gy), and (ii) a cohort of twenty healthy donors at two doses, 25 cGy and 2.5 Gy. *In vivo* radiation models included rodent, non-human primate, and radiotherapy patients undergoing total-body irradiation prior to bone marrow transplants. Our QRT-PCR data demonstrate diagnostically meaningful mRNA up-regulation for *BAX, GADD45a, DDB2,* and *CDKN1a* in all models. Dose response increases in the ratios of *BAX*/*BCL2* mRNA were also observed in our *in vivo* and *ex vivo* human and non-human primate models. Future studies will address preliminary data suggesting correlation between BAX and BCL-2 message and plasma protein levels in samples from BALBc mice irradiated *in vivo* at 0.25 Gy.

Combining *ex vivo* dose-response data with *in vivo* temporal gene expression studies have identified several coordinately responding genes altered by IR in diverse pathways. These findings permit the development, validation, and automation of higher (4-5 targets) multiplex QRT-PCR assays for forward-field laboratories. Our preliminary data suggest that these sentinel targets in QRT-PCR assays, when combined with other early medical parameters such as lymphocyte depletion kinetics, onset of vomiting, and protein bioassays, will be useful for assessing the extent of radiation injuries [AFRRI protocols BD-04 and BD-10, and an interagency research agreement with the National Institutes for Immunological and Allergic Diseases, NIH, Y1-AI-3823-01].

The Use of Discriminant Analysis for Evaluation of Early-Response Multiple Protein Biomarkers of Radiation Exposure Using Non-Human Primate 6-Gy Whole-Body Radiation Model

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The present need to rapidly identify severely injured irradiated individuals in mass casualty and population-monitoring scenarios prompted an evaluation of potential protein biomarkers that can provide early diagnostic information after exposure. Ionizing radiation induces time- and dose-dependent changes in gene expression and their respective protein products, which can be detected in blood cells. This work, which is a component of AFRRI's applied mission, is based on the central hypothesis that the level of specific proteins, measured using immunodiagnostic technologies, may be useful as protein biomarkers to provide early diagnostic information for acute radiation exposures.

Our research strategy involves the use of murine, non-human primate, and human models involving *ex vivo* and *in vivo* radiation exposure to identify and validate radiation responsive protein biomarkers. Using an *in vitro* model system of human peripheral lymphocytes as well as an *in vivo* murine model, we earlier reported radiation-responsive changes in the expression of the proto-oncogene proteins *ras*-p21 (Blakely *et al*., Proc. 36th Midyear Topical Meeting, Health Physics Society, pp. 231, 2003; Blakely *et al*., Adv. Space Res. 31(6): 1487, 2003) and, recently, *raf*-1, Gadd45, and DNA repair protein p21Waf1Cip1, each with a progressive time- and radiation-dose-dependent increase.

Here we present results from on-going studies using a non-human primate 6-Gy whole-body gamma radiation model. Protein targets were measured by ELISA in blood plasma before, 1, and 2 days after exposure. Data analyzed with use of multivariate discriminant analysis established very successful separation of non-human primates groups: 100% discrimination power for animals with correct classification for separation between groups before and 1 day after irradiation and 95% discrimination power for animals with correct classification for separation between groups before and 2 days after irradiation.

As expected these results demonstrate that multiple biomarkers provide enhanced discrimination of non-human primates with severe radiation exposure and injury. These results also demonstrate proof-in-concept that multiple protein biomarkers provide early diagnostic information to the medical community to effectively manage radiation casualty incidents.

Multiparameter and Integrated Biological Dosimetry — Protein Biomarkers and Biodosimetry Medical Recording Tools Supporting Radiation Casualty Incidents

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Effective medical management of suspected radiation exposure incidents requires the recording of dynamic medical data (clinical signs and symptoms), biological assessments of radiation exposure, and physical dosimetry in order to provide diagnostic information to the treating physician and dose assessment for personnel radiation protection records. The Biodosimetry Assessment Tool (BAT) is a comprehensive software application developed by the Armed Forces Radiobiology Research Institute (AFRRI) in collaboration with the Radiation Emergency Assistance Center/Training Site (REAC/TS) for recording diagnostic information in suspected radiological exposures (Sine *et al.*, Mil. Med. 166(12):85-87, 2001). The application, for use on the Microsoft Windows operating system, is available at the website www.afrri.usuhs.mil. The First-responder Radiological Assessment Triage (FRAT) is a complementary product for use on hand-held personal digital assistant devices. FRAT provides data collection templates for analysis of clinical signs and symptoms, lymphocyte counts, physical dosimetry, radioactivity, and location-based dose estimates. The FRAT application collects data in templates and compares it with known radiation dose responses to provide "triage" dose assessments. In the future, we envision upgrading our BAT application to permit downloading of FRAT data as well as providing additional tools to aid medical treatment decisions.

The need to rapidly assess radiation dose in mass casualty and population-monitoring scenarios prompted an evaluation of suitable biomarkers that can provide early diagnostic information after exposure. Hofmann and colleagues reported radiation-induced increases of serum amylase in 41 patients following either whole-body irradiation or irradiation of the head and neck region (Hofmann et al., Strahlenther Onkol. 166(10): 688-95, 1990). We recently investigated the utility of serum amylase and hematological markers (i.e., lymphocyte depletion) to provide early assessment of severe radiation exposures in a non-human primate model (i.e., Rhesus Macaques; n = 8) exposed to whole-body irradiation of 6.5 Gy ^{60}Co-gamma rays (40 cGy/min). Serum amylase activity was significantly elevated (12- and 2.5-fold of day zero samples) and lymphocyte cell counts depleted (≤20 % of day zero samples) one- and two-days after radiation exposure. These results demonstrate that protein biomarkers like serum amylase activity along with decreases of lymphocyte counts provide enhanced triage discrimination of individuals with severe radiation exposure and injury. [Acknowledgement: AFRRI, the National Institutes for Immunological and Allergic Diseases, National Institutes of Health (Bethesda, MD), and the Technical Support Working Group supported this research under work unit BD-02, BD-08, and BD-10, research agreement Y1-AI-3828-01, and Task 132D1 respectively.]

Improvement in the fabrication process of alanine pellet: influence on the angular response and fading

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The physic state of alanine mixed with other components in a pellet influences the shape of its spectra. It is obvious that the manufacturing process of pellet also influences the stabilization of the radicals produced by irradiation therefore its reproducibility.

As the alanine is an organic orthorhombic crystal, its orientation and thus radicals can be seen by the ESR measurement of the angular response. This work presents results about the angular response on pure alanine powders and its evolution during the manufacturing of homemade pellets.

Several pure alanines from different suppliers show different angular responses. Those evolve since the first step of the process (powder) until the granular form and also in the final form (pellet) after compacting. This latter influence partially the angular distribution, it is related to those observed before: granulation, drying, riddling. A step of reheating to improve mechanical properties of the pellets has also an influence. The final angular response has been also studied for pellets from different suppliers. Its evolution with time seems to be related with the others added components reputed as neutral for the alanine molecule. These observations contribute to the understanding on the alanine radicals stability.

Dose dependence of sensitivity of alanine radicals to visible light

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Early works on alanine dosimetry by Regulla and Deffner [1] showed, that dosimetric EPR signal in alanine decays after light illumination. Recent works by Ciesielski et al. [2,3] showed strong variations in the EPR spectra in γ-irradiated L-alanine exposed to fluorescent bulb, sunlight and even to daylight under normal laboratory conditions. They confirmed light sensitivity of the three different radical species R1, R2 and R3 and proposed R1→ R2 radical transformations for a plausible explanation of this effect. In this work we present results of our study of light-induced effects on radicals in L-alanine irradiated in a wide range of doses, from 0.5 kGy up to 4000 kGy. The source of visible light was made from 3 fluorescent bulbs (OSRAM, DULUXSTAR, 24W) mounted at 3 cm distance on both sides of a glass tray with alanine samples. Besides pure, crystalline L-alanine, the light effects on commercially available detectors: alanine pellets, films and rods were also studied. The light illumination of all types of alanine dosimeters resulted in decrease of the central EPR line from few percent up to 70%, depending on dose and type of the detector, and was accompanied by distinct variations in shape of EPR spectra. The degree of light-induced decay was found to be dependent on concentration of radicals induced in the detectors by ionizing radiation – in pure alanine powder the sensitivity was decreasing with increase of radical concentration and was lowest for 500 kGy samples, in which the EPR signal in non-illuminated detectors was maximal. Generally, in all detectors the decrease in amplitude of the central line was more pronounced than variation in total number of radicals reflected by double integral of the spectra. Analysis of those effects by numerical decomposition of the spectra into three components reflecting contributions of R1, R2 and R3 radicals showed, that decay in the R1 component was responsible for the observed decay of the dosimetric line. Observed increase in R2 contributions confirmed the suggested previously [2] R1→ R2 radical transformations. The reported effects should be taken into account in dosimetric procedures by introducing a necessity of protection of irradiated dosimeters from prolonged exposures to visible light.

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Study of various radicals proportions in simulated alanine spectra

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Alanine spectra revealed differences related to their external environmental storage conditions (hygrometry, temperature). It is due to different radicals proportions or their evolution with time. So, simulation ESR spectra of several single radicals issued from alanine radiolysis have been done. Those spectra have been mixed to identify the different areas that are the most sensitive to the radical proportions.

An angular response analysis of powder or pellets shows that the calculation based on one angle measurement is not accurate. Therefore, manufacturing a pellet with randomly oriented crystals seems unrealistic. Simulations have been done taking into account the angular response based on powder and single crystals for each single radical and their combination.

As several proportions of the three admitted radicals are still proposed, the deconvolution of the alanine spectrum and an evaluation of the dose response only regarding the major radical quantity to reduce uncertainties in ESR/alanine is an alternative. Before doing that, the proportions must be defined for each experimental condition and induces necessary to get some reference spectra.

Small radiation fields dosimetry with L-alanine and 2 methylalanine K-Band EPR miniature dosimeters

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With the new techniques of modern radiotherapy, such as: conformal radiotherapy (using multileaf collimators), IMRT, HDR brachiotherapy, gamma Knife, and radiosurgery with linear accelerator, the principal objective is to deliver the prescribed dose to the target volume with high spatial precision, minimizing the dose to neighboring normal tissues. In all of these treatment techniques the use of small radiation fields presents great difficulties. The challenge of a small radiation field in determining the prescribed dose to the target volume is the absence of lateral electronic equilibrium and the existence of a sharp gradient dose at the field border. Therefore, the dosimeter size is a very critical factor to perform small field dosimetry. The dosimeter size should be small enough to obtain high spatial resolution and then, to resolve the high gradient dose at the field border in beam profile (BP) determination. In the central part of a narrow beam, there is a small region where a uniform dose exists and out of it, the dose falls rapidly to zero with position. Consequently, a miniature dosimeter is necessary for correct normalization of dose in output factor (OF) measurements.

There are several techniques already applied to small field dosimetry, such as miniature ionization chamber, TLD, radiographic and radiochromic films, diamond, diode, liquid ionization chamber, MOSFET, etc. All of them have advantages and disadvantages. One technique that had not been entirely tested in narrow beam dosimetry is electron paramagnetic resonance (EPR) using alanine and/or 2 methylalanine (2MA) as a sensitive material.

The goal of this work was to test minidosimeters of L-alanine and 2MA in the determination of BP and OF measurements for small radiation fields. A K-Band EPR spectrometer (24 GHz) provides enough sensitivity to detect doses of the order of tens of grays. The results for BP and OF obtained with the L-alanine and 2MA minidosimeters were compared with others types of detectors such as ionization chamber, miniTLD and radiographic film. For example, the difference in OF between L-alanine and 2MA minidosimeters compared with miniTLD were 2,2% and 1,1% respectively for a 1x1 cm^2 field. In addition, the left side penumbra widths (10%-90%) for a 1x1 cm^2 field showed a difference of 1,7% and 0,3% for L-alanine and 2MA respectively compared with radiographic film.

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Improvement of sensitivity in ESR γ-dosimetry by gadolinium addition

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ESR dosimetry is an accurate method for photon and charged particle beams, useful both in radiotherapy and in the industrial application of ionizing radiation.

In the last decade to develop the applications of ESR dosimetry in the low dose range (~1Gy) the studies have been addressed toward signal analysis and toward the research of new materials with high radical yield and adequate intensity of ESR signal. In particular the studies on materials other than standard alanine have looked to find organic and inorganic compounds with better dosimetric properties, such as higher sensitivity and lower detectable dose, to extend the application of ESR dosimetry to radiotherapy. Among the various substances analyzed in these years a good dosimetric organic compound is ammonium tartrate. This substance offers greater sensitivity and lower detectable dose than alanine.

In this work we present the results obtained in analyses of ESR response of alanine and ammonium tartrate doped with gadolinium oxide exposed to ^{60}Co γ-photons. We have chosen gadolinium because of its large atomic number (Z=64). The interaction probability with photons (related to the interaction cross section) increases when the atomic number *Z* increases.

Solid state dosimeters of alanine were created with a blend of alanine, polyethylene, magnesium stearate and gadolinium oxide. Solid state dosimeters of ammonium tartrate were created in an analogous way.

The presence of gadolinium results in an improvement (by about a factor 2) in sensitivity of alanine and ammonium tartrate dosimeters to γ radiation and a reduction of the lowest detectable dose (which for the ammonium tartrate dosimeters doped with Gd was lower than 1 Gy).

EPR and TL dating of Dioptase (Chrysocolla) crystal

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The electron paramagnetic resonance (EPR) and thermoluminescence (TL) properties of natural crystalline Dioptase (Chrysocolla) from Brazil was investigated in order to understand the ionizing radiation effects on this crystal and to apply them in geological dating.

The crystal was powdered and irradiated at room temperature (γ-ray 60Co). The EPR measurements have shown the presence of E'1 centers in the natural samples. The intensity of this signal grows as a function of radiation dose. The accumulated dose Dac due to natural radiation was estimated using the additive method and it was evaluated to Dac = (1.44 ± 0.26) kGy. This result is larger than that obtained for the early study in phenakite [1]. An ICP-MS analysis was carried out finding high content of uranium (220 ppm), which gives an approximated annual dose of 26.8 mGy. The resulting age was about (54 ± 15) ka.

For comparison sake, TL measurements were carried out. The TL glow curves presented three peaks: 220, 330 and 420 oC (heating rate of 10 ºC/s). For dating, 330 ºC peak was used and the accumulated dose of (5.34 ± 0.96) kGy was obtained. Using the same annual dose above, the age of (200 ± 48) ka could be estimated.

The reason for this difference in the EPR and TL ages is under investigation. One possible explanation would be differences in the stability of the TL and EPR centers under exposure to UV light and heating, as was done for phenakite crystals.

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Lithium formate as a low-dose EPR radiation dosimeter

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The amino acid alanine has proven to be a highly accurate, precise and reliable dosimetry material for measuring doses of ionizing radiation using EPR-spectroscopy [1]. Nevertheless EPR/alanine dosimetry has its limitations when the dose of interest is in the order of a few Gy or less [2].

In the search for additional dosimetry materials which retains the excellent dosimetric properties of alanine, and in addition has a higher sensitivity than alanine, the lithium salt of formic acid, Lithium formate has shown promising properties [3-6]. Lithium formate has a sensitivity which is about 6 times higher than that of alanine, it is even more water equivalent than alanine (Z^{eff}=7.31), and the preliminary fading properties show very little fading in the first seven days. The EPR-resonance which is linear until above 1 kGy is mainly due to one radical which gives only one resolved EPR line. Recent works by Vestad et al. have demonstrated the potentials for accurately determining radiation doses down to below 0.1 Gy.

Some of the most important results of the studies of Lithium formate with respect to sensitivity, precision, stability and radiation quality dependencies will be reviewed in this presentation.

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The potential of Optically Stimulated Luminescence (OSL) of dental enamel for retrospective assessment of radiation exposure

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The Optically Stimulated Luminescence (OSL) technique using dental enamel may offer a fast and practical new method for retrospective assessment of radiation exposure if some of the technical challenges can be overcome. The OSL technique uses light to stimulate a radiation-induced luminescence signal from materials previously exposed to ionizing radiation. In general, this luminescence originates from radiation-induced defects in insulating crystals, and is proportional to the absorbed dose of radiation. The OSL technique has been successfully used for personnel dosimetry using high-sensitivity, artificially-grown crystals, and for sedimentary dating using natural crystals. It is well-known that the concentration of radiation-induced defects in hydroxyapatite from dental enamel has been successfully used for retrospective dosimetry using the EPR technique. The possibility of using OSL with dental enamel for similar applications was suggested by Godfrey-Smith and Pass (D. I. Godfrey-Smith and B. Pass, Health Physics 72, 744-749, 1997).

This work describes a preliminary assessment of the viability of using OSL with human dental enamel for retrospective assessment of radiation exposure. Investigations were performed with human dental enamel irradiated over a wide range of doses of beta radiation. OSL measurements were carried out using blue or infrared (IR) stimulation, and detection in the ulraviolet (UV) or visible region of the spectrum, depending on the stimulation light. Results are presented on the general characteristics of the main OSL (and thermoluminescence, TL) signals, and on the determination of optimum detection parameters including stimulation and detection wavelengths, signal stability, dose response, and estimation of the minimum detectable dose.

In our research conducted to date, an OSL signal from human dental enamel has been detected using both blue and IR stimulation from amounts of material of the order of a few milligrams (~1-5mg). Blue stimulation and UV detection seems to be the most appropriate combination in the sense that there is no signal from un-irradiated samples and the OSL decay is very clear. The minimum detection level achieved to date is about 10-20 Gy. Fading of the OSL signal was observed after irradiation. Work is now underway to improve detection capability. Some proposals will be discussed on the possible means to improve the viability of this technique.

Ultrasonic Analysis of Acute Thermal and Radiation Injury

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Medical injury from a terrorist event (IND, RDD) is likely to involve both radiation damage and thermal trauma (combined injury). A high frequency ultrasound technique has previously been developed at the Oak Ridge National Laboratory to function as a clinical tool to distinguish partial-thickness from full-thickness thermal burns in a porcine model and later extended for use in clinical burn units. A conventional ultrasound pulse-echo unit (5-30 MHz) was modified so that necrotic tissue at small distances (< 5 mm) from the skin surface could be resolved. Longitudinal resolution of soft-tissue damage is generally less than 0.1 mm. This requirement was necessary so that deep dermal damage could be separated from epidermal necrosis and from thermal injury near the subcutaneous fat layer. In a traditional clinical setting, the technique has shown sufficient sensitivity to quantitate extension of a partial-thickness burn to a full-thickness burn through cutaneous infection. The method uses a conventional B-scan unit with an adjunct computer-driven image frame grabber. Intensity and spectral analysis of the scattered ultrasound signal is performed through off-line computer image processing.

The technique described above has been extended in a pilot study to analyze radiation-induced cutaneous injury. The method uses a modified off-the-shelve medical B-scan unit operating at 10–30 MHz with online tissue characterization using image and spectral analysis. Analysis of radiation-induced skin injury is more difficult than for thermal injury, but enhancement of the technique has shown a time-dependent response curve for the scattered ultrasound signal after irradiation of Wistar rat tails to 40 Gy with a 120 KeV X-ray low LET spectrum.

The scattered intensity response curve peaks near the appearance of the first clinical sign (erythema) at 12 days post-irradiation. The mechanism of ultrasound sensitivity appears to involve changes in the tissue acoustic impedance due to hyperemia, vascular damage and leakage. Statistically significant changes ($p < 0.05$) in the magnitude of the reflected ultrasound spectrum have been noted less than 30 min post-irradiation. Because of the penetrating power and resolution of recent ultrasound equipment, this technique is expected to be extendable to analysis of irradiated deep organs, of large and medium-size blood vessels, and to possible analysis of combined injury.

New Probes for Environmental and Clinical Applications of EPR Spectroscopy

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EPR spectroscopy is specific to free radicals and paramagnetic molecules. It enables direct detection and quantification of free radicals in biological systems, *in vitro* as well as *in viv*o. In addition to being a valuable tool for characterization of free radicals, the EPR technique is also useful for examining tissue metabolism, oxygenation, redox state, perfusion, etc. This has led to increasingly widespread applications of the technique to complex problems involving physiology, pharmacology and pathophysiology in animal models. Although the EPR technology is by far limited to non-clinical applications, current developments in molecular probes and instrumentation may enable potential applications of EPR in the clinic. Among the types of measurements that are clinically important, measurement of tissue oxygenation (EPR oximetry) represents one of the most feasible applications.

'EPR oximetry' refers to the measurement of oxygen concentration by EPR spectroscopy. It requires the use of a probe (oximetry probe), whose EPR lineshape (mostly linewidth) is sensitive to molecular oxygen. EPR oximetry has advantages over the existing methods and enables reliable and accurate measurements of concentrations of molecular oxygen in tissues. Two types of probes are used: 'soluble probes' that report the concentration of dissolved oxygen and 'particulate probes' that measure partial pressure of oxygen in the milieu. The particulate probes offer certain advantages over the soluble probes including higher resolution, suitability for repeated measurements, non-invasive measurement, accuracy and reproducibility for localized measurements of oxygen. Naturally occurring (coals, fusinite), semisynthetic (India ink, sugar chars, carbon blacks) and purely synthetic materials (e.g., lithium phthalocyanine) have been reported for EPR oximetry. Single crystals of lithium phthalocyanine and its derivatives have received considerable attention in recent years.

We have developed new particulate oxygen probes based on the derivatives of lithium naphthalocyanine and evaluated their usefulness for biological oximetry. The new probes exhibit greater linewidth sensitivity to oxygen, high spin density, linear response to oxygen over a wide range of oxygen concentration, rapid response to changes in oxygenation, long-term stability in tissues, non-toxicity, and suitability for preparation of nanoparticulate suspensions for intravenous infusion for vascular imaging/oximetry, or internalization in cells to study cell migration, attachment, and proliferation. Implantable/retrievable formulations of the new probes offer potential clinical applications including cancer treatment wound healing, stem therapy, and peripheral vascular diseases. Some of these probes may also enable detection of exposure of trace amounts of certain environmental toxicants such as nitrogen dioxide and sulphur dioxide. The current status of development of these probes for potential clinical applications will be presented.

Technology Assessment and Roadmap for Emergency Radiation Dose Assessment

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A joint interagency working group (JIWG) under the auspices of the Department of Homeland Security Office of Research and Development conducted a technology assessment of emergency radiological dose assessment capabilities as part of the overall need for rapid emergency medical response in the event of a radiological terrorist event in the United States. The goal of the evaluation was to recommend general research and development needs to better prepare the Country for mitigating the effects of such an event. The general conclusions of the groups are: (1) Emergency radiation dose assessment by first responders is necessary to identify and focus the use of precious medical resources, to improve survival, and to enhance public confidence in government; (2) Radiation dose assessment is critically important because medical treatment depends on understanding the dose an individual receives; (3) Patients with very high radiation doses or significant internal contamination will likely present with no clinical symptoms other than possible conventional trauma; (4) Presently available methods are not satisfactory for managing the medical casualties from a radiological event and there is urgent need to develop new capabilities to assess radiation dose; (5) Dose assessment tools for use in triage and treatment are needed with a throughput of 1 assay per 5 minutes or less, and 24 hours or less for field-laboratories, with detection ranges of 1 – 8 Gy. Key thresholds to detect are 1.5 Gy and 4.5 Gy for triage. Treatment decisions thresholds to detect are 2 – 3 Gy and 6 7- Gy. There is also a need to identify those who *do not* need immediate medical attention. A research and development program focused on providing simple tools that can provide an estimate of whole or near whole body radiation dose is possible within the next 5 years. The goals of this program should be to: (1) Clarify device needs and requirements through a combination of user input, technology assessment and operations/systems studies; (2) Maximize use of existing technologies by concentrating near-term technology investments in developing pre-positioned dosimeter concepts and establishing a stable U.S. cytogenetics assessment network; (3) Pursue longer-range research and development to fill gaps with existing technologies through answering key questions about the throughput, specificity, prognostic value, sensitivity, range, accuracy and reliability of new radiation dose and injury assessment technologies; and (4) Conduct a demonstration program to assess the value of existing and proposed technologies. *This work performed under the auspices of the US DOE by the University of California, Lawrence Livermore National Laboratory under contract W-7405-ENG-48 with funding from the US Department of Homeland Security*

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